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Evaluation of Anti-Diabetic Activity of Polyherbal Combination of Carica Papaya and Curry Leaf

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ABSTRACT

The concerned study reveals antidiabetic effects of different polyherbal combinations of two medicinal plants used in traditional medicine. Aim of the present study was to evaluate antidiabetic action of polyherbal combination of two medicinal plants. Methanol: water (70:30) extracts of *Carica papaya* and *Curry leaf* were used for polyherbal combinations. All these combinations were studied for their acute toxicity and 200 mg/kg dose was selected. OGTT, antidiabetic activity and liver function tests were performed for all the combinations. Reduction in blood glucose level was determined in antidiabetic activity for 0 to 20 days and histopathology of the pancreas was performed after 20th day. Results revealed that all combinations were safe and dose was selected at 200 mg/kg. Polyherbal combinations II showed significant antidiabetic activity in OGTT and STZ-diabetic rats. Treatment with combination-II in diabetic animals produced beneficial improvement in lipid profile. Histopathological observations showed improvement in the rat treated with combination-II. It may be concluded that combination-II was most effective and safe in comparison to other combinations. Flavonoids, tannins and sterols present in this combination might be responsible for the effect.

Keywords: Polyherbal combinations; Acute toxicity; OGTT; Antidiabetic, STZ.

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INTRODUCTION

Diabetes mellitus is characterized by hyperglycaemia, hypercholesterolemia, and hypertriglyceridemia, resulting from defects in insulin secretion or reduced sensitivity of the tissue to insulin (insulin resistance) and/or combination of both[1]. The primary lesion at onset of diabetes, irrespective of the type, is a defect in insulin production and action, which is characterized by a clinical manifestation of hyperglycemia[2]. Although, there are numerous traditional medicinal plants reported to have antidiabetic properties. The medicinal uses of the plants used in the study are summarized in table 1[3,4].

It is well known that the incidence of diabetes mellitus is high all over the world, especially in Asia. Different types of oral antidiabetic agents such as biguanides and sulphonylurea are available along with insulin for the treatment of diabetes mellitus[5], but have side effects associated with their uses[6]. But more than three agents may be present in a medicinal herb with a variety of intervention targets via various mechanisms of action. Besides, polyherbal formulations[7] have proved more useful and beneficial in the management of various ailments including those that seem to defile conventional medication[8]. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Even the World Health Organization (WHO) approves the use of plant drugs for different diseases, including diabetes mellitus.

The potential role of the medicinal plants as antidiabetic agents has been reviewed by several authors, supported by the ethno botanical surveys and traditional medicines of different cultures[9]. Aim of the present study was to evaluate antidiabetic action of two commonly available medicinal plants in our region, *Carica papaya* (Caricaceae), and *Curry leaf* (Rutaceae). Present work was undertaken to find out best antidiabetic combination of the above mentioned commonly available herbs in our locality.

Table 1. Plants with their traditionally reported uses.

S.No	Plant name	Family	Uses
1	Carica papaya	Caricaceae	Antihyperlipidemic, Anticancer, hepatoprotective...etc
2	Curry leaf	Rutaceae	Body lotions, diffusers, potpourri, scent, air fresheners, body fragrance...etc

MATERIALS AND METHOD

Chemicals

Streptozotocin was obtained from Sacrum Research Laboratory, Mumbai, Maharashtra, India.

DPEC-GOD/POD kit for quantitative blood glucose determination was purchased from One Touch Horizon, India.

Extraction of Plant material

Leaves of *Carica papaya* and *Curry leaf* were collected from Chilakaluripet (Andrapradesh) and authenticated at Botanical Survey of India (Tirupathi). Dried powdered leaves of *Carica papaya* and *Curry leaf* were extracted by Sohxlet apparatus using Methanol: Water (70:30).

Animals

Wistar strain albino rats weighing between 215±15 g were obtained from the Albino research institute, Hyderabad. The rats were housed in clean metallic cages and kept in a well ventilated room and allowed to acclimatize to the laboratory condition for one week before being used. They were fed with standard animal pellet and had free access to water. The animals were distributed randomly into seven groups of six animals each for antidiabetic study using streptozotocin induced diabetic experiment.

Acute toxicity study

Healthy male Wistar rats, starved overnight (12 h), were divided into 24 groups of 6 each and were orally fed with increasing doses (50, 100, 250, 500, 1000, and 2000 mg/kg) of combinations I, II, III, and IV to determine the safe doses by up and down staircase method. The animals were observed continuously for one hour, then frequently for 4 hours, and later at the end of 24 h. After administration of the drug, Irwin test was conducted, where the animals were observed for behavioural changes. Further, animals were observed daily for 30 days, and mortality was recorded[10]. To know multiple dose toxicity of combinations, highest dose was fed once daily for 15 days and observed for incidences of mortality for a period of 30 days.

PHARMACOLOGICAL SCREENING

Oral glucose tolerance test in normal rats (OGTT)

Rats were divided into five groups (n = 6). They were fasted overnight and accessed to water only. Blood was taken from the lateral veins of the tail and the blood sugar levels were initially monitored with a glucometer (One touch Horizon). Above groups were treated with vehicle (0.5% Tween 80 solution), polyherbal combinations I, II, III, and IV (200 mg/kg, p.o., each). After 30 min, the animals were treated with 5% (wt/v) glucose orally. Blood glucose levels were monitored from lateral tail veins at 30, 60, and 120 min intervals after post glucose challenge[11].

Induction of diabetes mellitus

Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (35 mg/kg; bw) in 0.1M citrate buffer (PH 4.5) to overnight fasted rats. The development of diabetes

was confirmed after 48 hours of STZ injection, the animals with fasting blood glucose level more than 200 mg/dl were selected for the experimentation[12].

Experimental Design

The Streptozocin-induced diabetic Wistar rats were randomly assigned into six groups (1-6) of six rats (n=6) each. Group 1 received normal saline p.o., group 2 received streptozotocin (35 mg/kg, i.p.), group 3 received Metformin (200 mg/kg, p.o.), group 4 received Combination I (200 mg/kg, p.o.), group 5 received Combination II (200 mg/kg, p.o.), group 6 received Combination III (200 mg/kg, p.o.), and group 7 received Combination

IV (200 mg/kg, p.o.)

Determination of blood glucose levels

Blood samples were collected by cutting the tail-tip of the rats, for blood glucose determination at intervals of 0, 5, 10, 15, and 20 days. Determination of the blood glucose level was done by the glucose-oxidase principle using the ONE TOUCH Basic (Horizone) instrument and results were reported as mg/dl[13].

Biochemical estimation

Blood glucose level (BGL), total cholesterol (TC), high density lipoprotein (HDL) cholesterol, triglycerides (TG) were estimated using standard kits of Bayers diagnostic Pvt. Ltd., India. Low density lipoprotein (LDL)-cholesterol was calculated from the measurement by Friedwald formula[14].

Histopathology

On 20st day the animals were sacrificed, the pancreas of one animal from each group was excised and stored in 10% formalin after washing with normal saline. Histopathological parameters were studied at Omega laboratory, Lonand, Satara, India. The tissue was washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial sections of 5 µm thickness were cut using a rotary microtome. The sections were then deparaffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to haematoxylin for 10 min, followed by rinsing with water. These were examined and later counterstained with esion, rinsed with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted.

Statistical analysis

The data was analyzed by one-way ANOVA followed by Tukey-Kramar multiple comparison test. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Experiments were carried out on normal healthy rats for acute toxicity studies. The behavior of the treated rats appeared normal. No toxic effect was seen even with the dose of 2.5 g/kg b.w. and there were no lethality in any of the group. Body weight was normal.

Experiments were carried out on normal healthy rats for acute toxicity studies. The behaviour of the treated rats appeared normal. No toxic effect was seen even with the dose of 2.5 g/kg b.w. and there were no lethality in any of the group. Body weight was normal. Therefore, the cut off dose for effective dose (ED50) was taken as 200 mg/kg b. w. which is the 1/10th of LD50. All the two plants selected were having reported hypoglycemic and anti-diabetic activity. Methanol: water(70:30) extract of *Carica papaya* and *Curry leaf* were reported as good antidiabetic agents (Table 1). So these extracts were used for preparing suitable active combinations I to IV.

The oral glucose tolerance test results showed that the plants extracts combinations showed some antidiabetic effects on the blood glucose level in the fasting normal rats. The critical test for diabetes does not lie in hyperglycemia or hyperlipidaemia but in blood-sugar tolerance. After ingesting sugar, both normal and diabetic individuals will show an increase in the blood sugar level as it happens after a meal, but the increase remains high in the diabetic, whereas in the normal individual the excess glucose is rapidly converted into glycogen. Polyherbal combination-II among different formulations was observed to be most active in lowering the postprandial blood glucose level (Table 2). This may be due to synergistic effects of the chemical constituents of the two plants and shows a great promise as an oral antidiabetic agent.

Table 2. Antidiabetic effect of various polyherbal combinations in OGTT

Treatment	**Mean blood glucose concentration (mg\dl)**			
	0 min	**30min**	**60 min**	**120min**
Control	112 ± 2.0	255 ± 2.3	285 ± 2.3	158 ± 2.1
Combination I	107 ± 1.6	250 ± 2.6	141 ± 2.1	122 ± 1.9
Combination II	102 ± 3.2	225 ± 3.6	121 ± 2.5 *	116 ± 1.5 *
Combination III	106 ± 2.6	215 ± 3.4	188 ± 2.1 *	131 ± 1.8 *
Combination IV	103 ± 2.8	192 ± 2.1 *	140 ± 3.2 *	120 ± 2.9 *

Results are expressed as Mean ± S. E. M. (n = 6); * = p<0.05 compared with control.

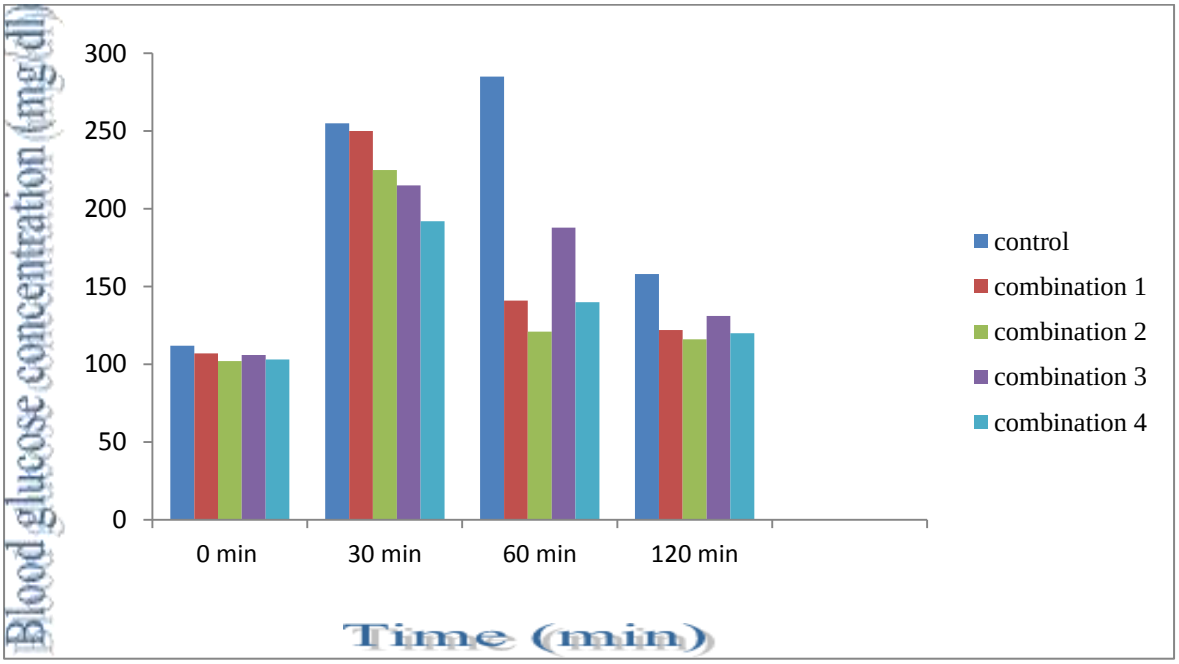

Figure 1: Antidiabetic effect of various polyherbal combinations in OGTT

The results of the effects of various polyherbal combinations (200 mg/Kg, p.o.), Metformin (200 mg/Kg, p.o.), and control groups in streptozocin-induced diabetic Wistar rats. A drop of blood samples was collected by cutting the tail-tip of the rats, for the blood glucose determination at intervals of 0, 5, 10, 15 and 20 days. All the polyherbal combinations showed significant (p<0.05) reduction in blood glucose level but combination II was found to be better amongst all in reduction of blood glucose level.

Table 3. Effect of various polyherbal combinations on streptozotocin-induced diabetic wistar rats

Treatment	Blood glucose concentration (mg\dl)				
	0 day	**5 day**	**10 day**	**15 day**	**20 day**
Control	110.89 ± 3.80	112.64 ± 2.8	110.80 ± 3.5	114 ± 2.16	115.64 ± 2.8
Disease control	289.20 ± 6.73	267.40 ± 5.0	285.20 ± 8.2	279 ± 1.1	281 ± 5.23
Metformin	315.60 ± 4.8	256.81 ± 4.3	182.82 ± 5. 3 *	138 ± 3.71 *	118 ± 2.45 *
Combination I	310 ± 1.0	255 ± 1.25	195 ± 1.2 *	152 ± 3.5 *	130 ± 1.78 *
Combina	292 ± 3	178 ± 1.	151 ±	132 ± 3.	115 ± 2.

tion II	. 7 4	2 *	2 . 6 5 *	1 *	5 8 *
Combina	3 0 6	2 1 0 ± 4 .	1 7 2 ± 7 .	1 4 1 ± 1 .	1 3 2 ± 5 .
tion III	± 3 . 6	2 5	3 *	6 5 *	6 *
Combina	3 2 8 ± 4	2 9 0 ± 5 .	1 9 8 ± 1 .	1 6 2 ± 5 .	1 4 1 ± 2 .
tion IV	. 6 5	2	6 5 *	1 *	6 *

Vales are given as mean ± SEM for 6 rats in each group; experimental groups are compared with diabetic control. Values are statistically significant at *=P<0.05.

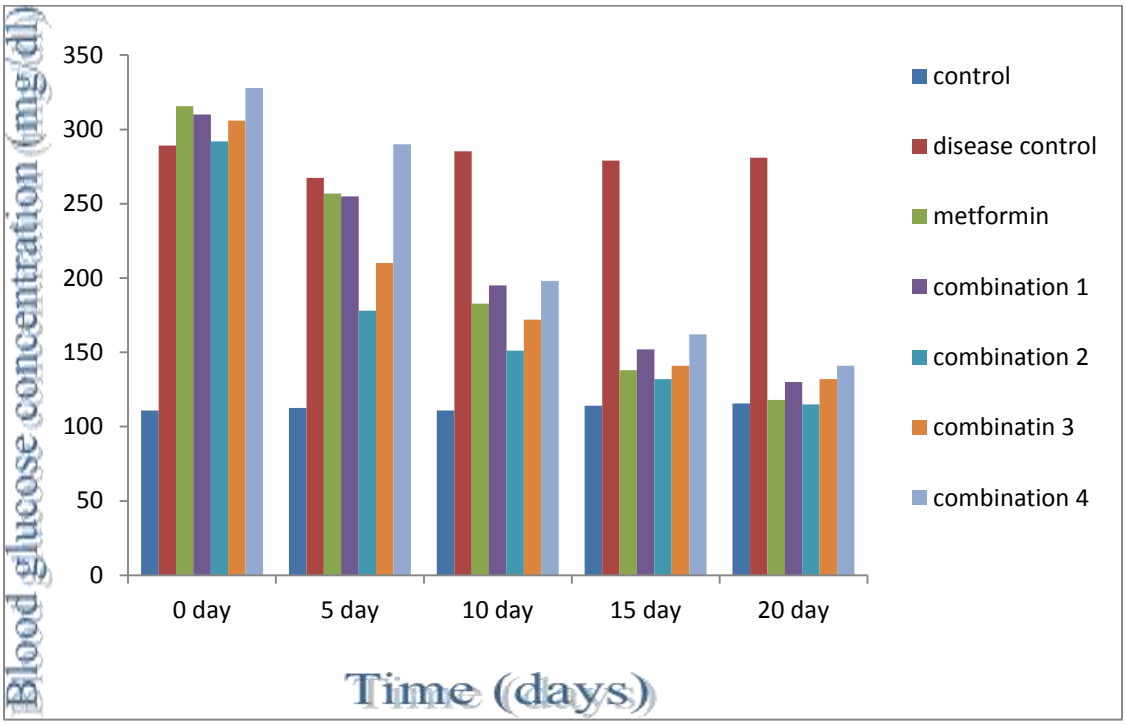

Figure 2: Effect of various polyherbal combinations on streptozotocin-induced diabetic wistar rats

The diabetic control animals showed significant increase in total cholesterol, serum LDL-cholesterol and serum triglycerides level compared to control animals. Total cholesterol, serum LDL-cholesterol and serum triglycerides levels in combination-II treated diabetic rats showed significant decrease ($p < 0.05$) than other combinations compared to diabetic rats. Results are compared with standard drug metformin. Serum HDL-cholesterol level was significantly decreased in diabetic rats compared to control rats. Diabetic rats treated with combination-II showed significant ($p < 0.05$) increased in HDL-cholesterol than other combination compared to diabetic animals (Table 4).

Table 4. Effect of various polyherbal combinations on total cholesterol, LDL, HDL and serum triglycerides.

Treatment	Total	LDL	HDL	Serum Triglycerides

	Cholesterol(mg\dl)	**(mg\dl)**	**(mg\dl)**	**(mg\dl)**
Control	111.69 ± 5.4	46.42 ± 5.0	42.99± 2.6	138.0± 3.5
Disease control	163.4± 3.64	101.5± 5.3	23.67± 1.9	185.0± 3.9
Metformin	122.7± 3.80*	57.54± 5.2*	38.75± 3.0*	151.0± 2.9*
Combination I	147.8± 2.80	85.36± 3.5	37.74± 3.2	173.0± 2.1
Combination II	123.3± 3.46*	60.28± 2.1*	32.74± 3.3*	150.1± 3.0*
Combination III	141.8± 4.17*	72.25± 3.1	35.75± 4.0*	167.6± 3.2*
Combination IV	152.2± 3.87	88.47 ± 2.7	33.12 ± 3.1	177± 5.1

Vales are given as mean ± SEM for 6 rats in each group; experimental groups are compared with diabetic control. Values are statistically significant at *=P<0.05.

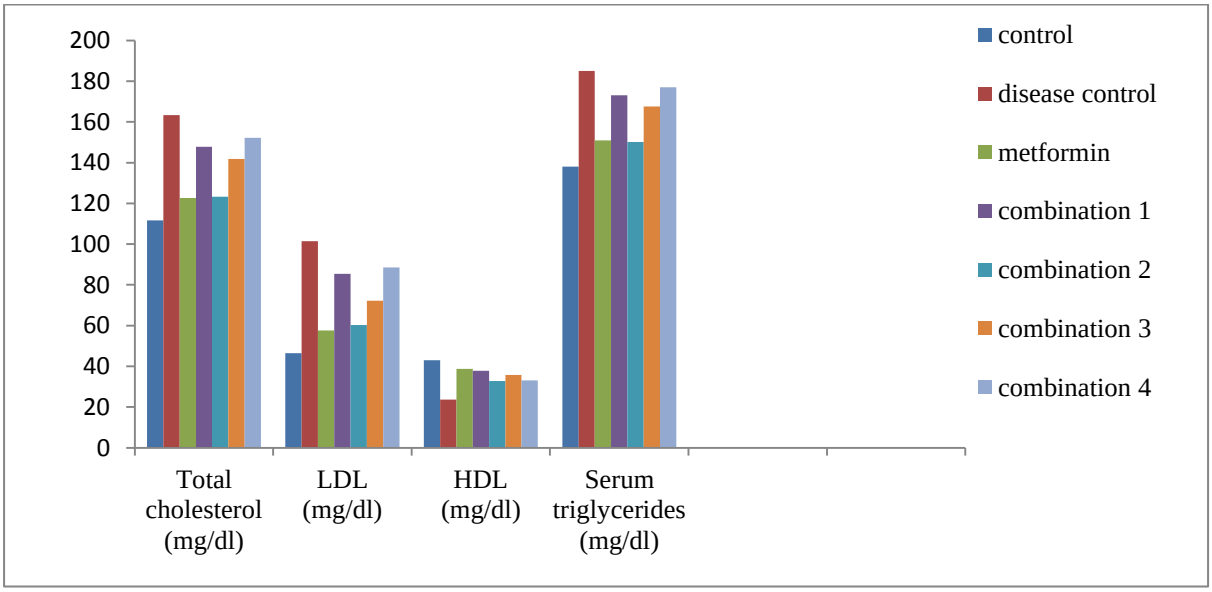

Figure 3. Effect of various polyherbal combinations on total cholesterol, LDL, HDL and serum triglycerides.

Streptozocin-induced hyperglycaemia has been described as a useful experimental model to study the activity of antidiabetic agents. Streptozocin selectively destroyed the pancreatic insulin secreting β cells, leaving less active cell resulting in a diabetic state. The test samples might possess metformin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. The phytochemical studies of extracts of polyherbal combinations revealed the presence of tannins, carbohydrate, terpenes, saponins, and flavonoids. Flavonoid and terpenes possess antidiabetic action. Effect of the flavonoids on pancreatic β-cells leading to their proliferation and secretion of more insulin by which they reduced hyperglycaemia caused by streptozocin in diabetic rats. These secondary metabolites present in poly herbal combinations may also be acting similarly thereby decreasing the high blood glucose levels of streptozocin-diabetic rats.

In present study elevated serum total cholesterol, triglycerides, LDL-cholesterol, reduced-HDL-

cholesterol was observed. Treatm-ent with combination-II in diabetic animals produced beneficial improvement in lipid profile. In the diabetic Control, decrease of pancreatic islet numbers and their size, atrophy and vacuolation and invasion of connective tissues in parenchyma of pancreatic islets were detected but these abnormal histological signs were dramatically decreased in combination-II dosing groups compared to that of control. Similar histopathological changes of the pancreas were observed in metformin dosing group.

CONCLUSION

It may be concluded that combination-II was most effective in comparison to other combinations and Metformin and there were no toxic effects during the 7 hr of study. Combination-II has shown remarking effect on blood glucose level and marked improvement on hyperlipidemia due to diabetic. Its specific effect on HDL has additional advantage in checking coronary risks.

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