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Study of normoglycemic and antihyperglycemic effect of *Vinca rosea* extract in alloxan treated diabetic mice

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Abstract : The Diabetogenic agent, Alloxan, like ROS form superoxide radicals by the Fenton reaction, causes massive concentration of Ca^{++} in cytosol of β-cell and its dysfunction. The proven potential of *Vinca rosea* extract against Cancer can be equally effective against Diabetes mellitus, with less antigenicity when taken orally. In 150 minutes of study, each at the interval of 30 minutes, the normoglycemic role of methanolic extract of *Vinca rosea* @ 300mg/kg body weight and 500mg/kg body weight were observed and analysed After giving a dose of sugar solution@3mg/kg body weight to each mice, the treated group had an increase of only 10.4%blood glucose level after taking *Vinca rosea* extract @ 300mg/kgbw, whereas untreated group exhibited 146% increase in blood glucose level.

After 21 day of study, antihyperglycemic effect of *Vinca rosea* extract @300mg/kgbw and 500mg/kgbw were observed in reference to the normal control mice group without assess to *Vinca rosea* extract and diabetic control group of mice without assess to *Vinca rosea* extract. The dose @300mg/kgbw reduced blood glucose level by 68.5% and the dose @500mg/kgbw reduced blood glucose by 87.3% without any adverse effect on body weight and parameters of the LFT, KFT and PFT.

Key words: Alloxan diabetic mice, *Vinca rosea extract*, ROS, Fenton reaction, LFTs KFTs, PFTs.

INTRODUCTION

Diabetes mellitus is one of the most common chronic endocrine metabolic disorders in approximately 10% of the world population with micro and macro complications that result in significant morbidity and mortality. Due to side effects associated with the use of Insulin and its hypoglycemic effect many natural plant products and bitter tasting foods are used for managing diabetes. Since time immemorial, patients with non- insulin dependent diabetes mellitus have been treated orally by folklore with a variety of plant extracts.

According to the WHO, 150 Millions people have diabetes world wide and this number may well double by the year 2025. The prevalence of increase in diabetes is observed due to the population growth and ageing with sedentary life styles, having little or no exercise, unhealthy diets and being overweight etc.

Presently, India is supposed the diabetes capital of the world, since over 40 million diabetes sufferers are alone in India and that by 2025 that number will grow upto 70 million. In other words, 1 of every 5 diabetics in the world will be an Indian. Diabetes is the number one cause of kidney failure is responsible for 5% of blindness in adults and 1 million limb amputations.

Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) is an oxygenated Pyrimidine derivative present as Alloxanhydrarte in aqueous solution. Alloxan is a toxic glucose analogue, which selectively destroys

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insulin-producing cells in the pancreas (beta cells). Lenzen,S. *et.al* (2008) found the detailed mechanism of inducing diabetes through Alloxan & Streptozotocin, they found the mechanism mediated through alloxan is by release of reactive oxygen species as free radical. Streptozotocin produce fragment of DNA in β-cell & inhibiting it glucose induce insulin secrection.

Miranda *et al (*1978) have shown that diets supplemented with high fiber content reduce blood sugar level. Similar results were obtained from Doughlas *et al* (1975) that dietary fiber and fiber analogue reduce the blood sugar level.

Ayurvedic herbal medicines have been shown to produce hypoglycemic action in diabetic patients. Chattopadhyay RR, Sarkar SK, Ganguly S , Banerjee RN, Basu T K (1992) studied the effect of extract of leaves of *Vinca rosea* Linn. on glucose utilization and glycogen deposition by isolated rat hemi diaphragm the water soluble portion of alcoholic extract of *Vinca rosea* leaves has been shown to possess significant hypoglycemic and antihyperglycemic effect in rats.

Chattopadhyay RR, Sarkar SK, Ganguly S , Banerjee RN, Basu TK (1992) studied the hypoglycemic and antihyperglycemic effect of leaves of *Vinca rosea Linn*. Extract of *Vinca rosea* led to marked lowering of blood glucose level in normal and streptozotocin induced diabetic rats. The hypoglycemic effect of the fraction was comparable with that of tolbutamide.They also studied the anti-inflammatory and acute toxicity studies against carrageenan induced rat hind paw edema.

Chopra *et al* 1956 mentioned that *Vinca rosea* is a medicinal plant commonly grown in Indian garden and a native of West Indies. Parts of this plant have been reported to have medicinal properties for antidiabetic, antileukemic and hypotensive activities. Studies of Singh, S.N. *et al* reported that the extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats increases metabolization of glucose.

Nammi,S. *et al* observed that the blood glucose lowering effect of leaf juice of *Catharanthus roseus* in normal & diabetic rabbit comparable with the standard drug glibenclamide. It is probably mediated through enhance secreting of insulin from β-cells of Langerhans.

Asthana *et.al* (1979), Chopra R.N *et.al* (1956), Sumana G *et.al* (2001), Grover JK *et.al* (2002), Gupta (2008) & others also concluded the stress regenerated diabetes has been subsided by use of *vicia rosea* extract.

MATERIALAND METHODS

Fresh and young whole plants of *Vinca rosea* were collected from Biodiversity Garden of the Department of Biotechnology, A.N. College campus. These were washed with sufficient water to free from earthly impurities. The washed plants were then dried under shade for one week. The dried leaves were then grinded to coarse powder. One kilogram of coarse powder of *Vinca rosea* was used for extraction with methanol in soxlate apparatus and extraction process used about 30 hours continuously with many siphoning. The methanolic extract was evaporated to dryness through Rotavapour (Buchi Company) under reduced pressure and low temperature. The remaining plant extract was collected (15.5%w/w) from the round bottle flask and preserved in deep freezer for experimental tests. The albino mice were available in the department by continuous breeding programms. For experimentation adult mice weighing 30-40 gm were selected.

The albino mice have been chosen here for the study of the effects of Alloxan on β-cell of pancreas and then the efficacy of medicinal plant *Vinca rosea.*

Blood samples were obtained from mice by cardiac puncture and tail vein puncture. In diabetes, the experiments were designed in two ways for studies-

1. Dose Dependent Experiments
2. Days Dependent Experiments

In the present study, the experiments were designed through Days Dependent Experiment where Dose per Kg body weight give better results in mice models because the doses administrated give direct impact on them. During the present study the mice have been divided into five groups and one control. Sacrifices were made every 1st week and 4th week of the treatments with *Vineca rosea* extracts. Data were collected and analyzed statistically.

RESULTS & DISCUSSION

Test of Hypoglycemic effect- Blood sugar in the case of Euglycemic mice remains fairly constant that was 1 mg/ml. After food intake, there is a temporary rise in the blood glucose level at half an hour duration depending upon the type of food taken. Blood sugar level returns to normal within two or three hours after taking food. It

was observed that in decreased glucose tolerance, blood sugar level does not returns to normal within two to three hours after food intake.

To study the hypoglycemic property- Methanolic extract of *Vinca rosea* was used for experimentations. Albino mice were divided into six groups, each group with eight individuals were acclimatized for this test.They were fasted for 24 hours before the following administrations were given-

Group I- Received only distilled water

Group II- Received glucose solution 3mg/kg

Group III- Received methanolic extract 300 mg/kgbw with glucose solution

Group IV- Received *Vinca rosea* extract 500mg/kgbw along with glucose solution

Group V- Received only *Vinca rosea* extract 300mg/kgbw

Group VI- Received only *Vinca rosea* extract 500mg/kgbw

Group II, III and IV were loaded with glucose 3mg/kgbw in aqueous form orally, half an hour after the *Vinca rosea* extract treatments. Blood samples were collected by puncturing the tip of tail just before any administration as well as 30, 90 and 150 minutes after giving glucose load. Blood plasma sugar level was measured using Electronic glucometer. The data obtained were as given in the table :1

The effect of *Vinca rosea* extract in the doses of 300mg/kgbw and 500mg/kgbw on glucose tolerance test in comparison to control mice which were given only distilled water to same amount of extract to equalize the impact on plasma concentration were recorded after just before dose, 30 minutes after dose, 90 minutes after dose and 150 minutes after dose and significance test were calculated after each experiment and each groups by periusal of table it was clear that methanolic extract of *Vinca rosea* has significant effect in reducing plasma glucose level.

Table: 1. Plasma glucose level (mg/dl)

	Just before any dose	30 minutes after the dose	90 minutes after the dose	150 minutes after the dose
Group I	72±3.6	72 ±2.8	71 ±3.4	71± 5.9
Group II	66±2.8	121 ±5.6	100 ±5.9	98 ±6.2
Group III	65±2.6	92 ±3.6	60± 4.2	60 ±3.2
Group IV	68±2.9	90 ±3.4	64 ±3.2	65 ±4.3
Group V	67±2.5	60 ±4.3	58 ±5.2	60 ±3.2
Group VI	69±3.1	58 ±2.6	55± 4.3	59 ±2.7

To study the antihyperglycemic property- The antihyperglycemic property of *Vinca rosea* extracts were studied with the help of six experimental groups. Group I (normal control group) which had no assess to any nutrients till the experimental processes, and another, Group-II (diabetic control group) which were supplied with a glucose load of 3g/kgbw in aqueous form. Group III and Group IV were constituted to assess whether the hypoglycemic action goes on Euglycemic individuals with minimum blood glucose level or not. Group I mice exhibits the glucose homeostasis by keeping the Blood glucose level at 71 to 72mg/dl. Group II mice exhibited the overshoot of blood glucose level which double in the treated with glucose load. After few hours the blood glucose level returns to the normal level. Mice of Group III and Group IV of the table exhibit no significant hypoglycemic effect at the dose of 300mg/kgbw in Euglycemic animals but 500mg/kgbw of *Vinca rosea* extract dose results in significant hypoglycemic effect. The best hypoglycemic result is observed at higher dose after 90 minutes of dose given in both groups of animals with glucose or without glucose load. Group II mice were given the glucose load to assess the pattern of Blood glucose level with reference to the time frame under consideration. The blood glucose level arose up to 121mg/dl from 66mg/dl, which is approximately double. The same parameter returns towards the normal value 98 mg/dl. Studies of Group III and Group IV indicate the normoglycemic effect on no diabetic individuals which were treated either by 300mg/kgbwor500mg/kgbw.

The table:2 exhibits no significant hypoglycemic effect at the dose of 300mg/kgbw in Euglycemic animals but 500mg/kgbw of *Vinca rosea* extract dose results in slight degree of hypoglycemic effect. Group III and Group IV results were more vital which confirms the rapid glucose homeostatic effect by *Vinca rosea* extract. Since the *Vinca* rosea extract were administered half an hour before the glucose load given for the first time hence the maximum rise in blood glucose level Fluctuation in Glucose level was more prominent in mice groups without *Vinca rosea* extract administration. Mice of group III and group IV when given the *Vinca rosea* extract, got activation of the antihyperglycemic machinery, hence the blood glucose level not overshoot even after the glucose load introduced as compared to the Mice of group II which also had given glucose load.

The data obtained indicates the potentializing effect of *Vinca rosea* extract to insulin or glucose utilizing machinery. Murthy, P.S (1995) found the potentializing effect of plant extract including *Vinca rosea* in diabetes. But the effect of *Vinca rosea* extract could not become active on the mice whose blood sugar level was at threshold level. Table :2 indicates effect of *Vinca rosea* extract in normal and diabetic groups of mice.

Table: 2. Data showing effect of *Vinca rosea* extract on different parameters

	1st day	7th day	14th day	21st day	
Normal control	121.23	120.6	125.3	121.6	ALP(K.A.units/dl)
	0.56	0.55	0.55	0.54	S.Bilirubin(mg/dl)
	7.53	7.5	7.48	7.40	Total protein(g/dl)
	0.53	0.52	0.50	0.52	Creatinine(mg/dl)
	51.2	52.0	52.3	51.8	Lipase(mg/dl)
	32	35	38	39	Body weight(gm)
Diabetic control	280	285	288	290	ALP(K.A.units/dl)
	0.42	0.40	0.38	0.37	S.Bilirubin(mg/dl)
	6.7	6.1	6.0	6.0	Total protein(g/dl)
	2.4	2.3	2.6	2.9	Creatinine(mg/dl)
	91.3	95	96	96	Lipase(mg/dl)
	36	30	28	27	Bodyweight(gm)
Treateddiabetic (300mg/kgbw)	260	180	160	145	ALP(K.A.units/dl)
	0.45	0.48	0.51	0.53	S.Bilirubin(mg/dl)
	6.8	7.0	7.0	7.1	Total protein(g/dl)
	2.0	1.6	1.1	0.6	Creatinine(mg/dl)
	89	70	69	69	Lipase(mg/dl)
	32	40	42	43	Bodyweight(gm)
Treated diabetic (500 mg/kgbw)	240	150	140	136	ALP(K.A.units/dl)
	0.45	0.48	0.51	0.53	S.Bilirubin(mg/dl)
	6.8	7.0	7.0	7.1	Totalprotein(g/dl)
	2.0	1.6	1.1	0.6	Creatinine(mg/dl)
	85	68	66	65	Lipase(mg/dl)
	33	32	31	32	Body weight(gm.) y wwwwtweight(gm

CONCLUSION:

Oral AdmOral administration of methanolic extract of *Vinca rosea* @ 300mg/kg body weight and 500mg/kg body weight were effective as days and dose dependent experimentations which help in lowering the blood glucose level in only diabetic group of mice, without any significant toxicity in functions of Pancreas, Liver or Kidney. The results indicate a prolonged action in reduction of blood glucose by *Vinca rosea* and its mode of antidiabetic action of the active principle may be probably either through activating the enhance insulin release machinery from β-cells of Langerhans or any other extra pancreatic factor.

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