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Glycemic interaction of aqueous extract of *Vinca rosea* in albino mice

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Abstract : In the present research work, aqueous extract of *Vinca rosea*[L] proves to be hypoglycemic and anti diabetic without any significant adverse effect on liver. In an one month long aqueous extract treatment, no antigenic effect was reported against the markers studied were SGPT, Alkaline Phosphatase and Serum bilirubin for Liver function test in both fasting and post prandial cases. The p- value of Serum bilirubin become less than 0.1 ,p- value for SGPT remain less than 0.1 , p- value for Alkaline phosphatase less than 0.02 for extract treatment in euglycemic albino mice. The hypoglycemic effect of aqueous extract was non significant ($p>0.1$) after 10 days but in overall studies it's p- value remained less than 0.001 in fasting subjects and similar p-value for post prandial subjects. The gradual action of the extract is indication for inducing insulin secretion.

Key words: Hypoglycemic, SGPT, Alkaline phosphates, Serum bilirubin, antigenic

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by Hyperglycemia, Glycosuria, Negative nitrogen balance and Ketonurea along with causing a number of complications like Retinopathy, Neuropathy and Peripheral vascular insufficiencies etc. The Chronic metabolic disorder that affects 150 million people is set to rise more than 300 million by 2025 as per WHO report.

Diabetes mellitus is classified as –

Type I diabetes (IDDM- Insulin Dependent Diabetic Mellitus) is an auto immune genetic disease resulting from and absolute deficiency of insulin due to destruction of insulin producing pancreatic β-cell.

Type II diabetes (NIDDM-Non Insulin Dependent Diabetes Mellitus) is multi factorial disease which is characterized by insulin resistance associated not only with Hyperinsulinanemia and Hyperglycemia but also with Atherosclerosis, Hypertension and abnormal lipid profile collectively called syndrome-X. It accounts 90-95% of diagnosed cases of this disease.

India has a tremendous wealth of medicinal plants. Still exhaustive investigations are required to take out the hidden secretes for cerements of various diseases in general and diabetes in particular.The hypoglycemic products from plant sources are identified as flavonoids, glycosides, alkylsulfides, xanthones and peptides etc.

Ghosh and Gupta (1980)[4] & Chattopadhaya*et al*. (1999)[2] observed that aqueous extract of *Vinca rosea* leaves reduce blood glucose upto 20 % where as ethanolic extract reduces sugar by 49-58%.

Singh *et al*. (2001)[9] observed that the rats pretreated with alcoholic extract were completely immune to the diabetes where as aqueous extract has minor preventive effect.

Many researcher reported that administration of aqueous extract of *Vinca rosea* leaf have been found to regulate the glucose level in alloxan diabetic male albino rats and also reversed the changes in carbohydrate, lipid, protein and metabolites as well as other parameters of pancreas , liver and kidney. The aqueous extract of *Vinca*

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rosea led to significant lowering of blood glucose level and reduction in serum lipid was also reported.

MATERIALS & METHODS

1. Selection of diabetic mice

Diabetes weas induced by intraperitoneal injections of alloxan in freshly prepared normal saline solution injecting 150mg/kg/day for 3 successive doses in mice fasted overnight. The control mice also received the same value of saline. Blood samples for measurement of diabetic parameters were obtained after 3 days of final alloxan injection .Lazarow (1964)[8], in his experiment estabilished the mechanism of β-cell destruction by use of chemicals like Alloxan, producing free redicals.

2.Preparation of leaf extract-

Fresh leaves of *Vinca rosea* were collected from Biodiversity Garden of the Department, Biotechnology, and A.N.College campus. It was water washed, shade dried and then coarsely powered. Aqueous extract was obtained throughSoxhlet apparatus for 3 days of continuous running. The aqueous extract filtered with funnel was converted to gel by low pressure treatment in Rotavapour (Buchi Company).

3.**Induction of aqueous extract in diabetic and control mice-**

Aqueous extract of *Vinca rosea*was intraperitoneally introduced in mice @ 200mg|kg body weight.

4.**Obtaining experimental data-**

(a)Estimation of Blood Parameters recorded through Autoanalyzer (Vital scientific, Merck).

(b) Detection of alkaloids in extract of leaf- The extract of leaf was digested with 2M HCl. The filtrate was mixed with amyl alcohol. The appearance of pink colour indicated the presence of alkaloids.

5.Grouping of mice for treatment:

For the present research work 10 normal Euglycemic mice (group I) and 20 Alloxan-diabetic mice (group II), each weighing 30-40 gm, of one month age were selected for experimentation. Group I- Euglycemic normal mice who were on diet control and given *Vinca rosea* extracts (10 cases). Group II- Alloxan diabetic mice were kept on diet control and given *Vinca rosea* extract (20 cases). All alloxan diabetic mice and normal mice were investigated individually for blood glucose in fasting and post prandial states, for twice -after one hour and after two hours of giving meal. Extract of *Vinca rosea* was administered half an hour before meal daily for the entire period of study. The blood glucose level was estimated on 1st, 10th, 20th and 30th day of study. Each time it was estimated in fasting

Table-I Laboratory investigations for Group-I case: Blood glucose estimation (mg\dl).

10 N	Onset			After 10 days			After 20 days			After 30 days		
	F	1hr	2 hr	F	1 hr	2 hr	F	1hr	2hr	F	1hr	2hr
1.	78	126	96	75	124	94	72	122	92	68	120	89
2.	74	128	96	70	128	92	68	124	90	64	120	84
3.	82	120	118	78	118	114	76	114	112	74	110	108
4.	76	122	94	74	120	92	72	116	88	68	114	84
5.	80	122	98	78	120	96	74	118	94	70	116	92
6.	84	120	112	84	118	110	80	112	110	82	112	108
7.	72	116	94	72	114	94	74	114	92	72	112	92
8.	74	116	98	74	114	96	72	114	94	72	112	94
9.	82	128	112	80	128	110	80	126	110	78	126	108
10.	88	132	120	84	130	116	82	128	114	80	126	110
Mean	**79**	**123**	**103.8**	**76.9**	**121.4**	**101.4**	**75**	**118.8**	**99.6**	**72.8**	**116.8**	**96.9**
S.D.+_	**05.1**	**05.35**	**10.43**	**4.77**	**5.82**	**9.8**	**4.45**	**5.75**	**10.45**	**5.75**	**5.9**	**10.5**
P- value	**-**	**-**	**-**	**>0.1**	**>0.05**	**>0.1**	**<0.02**	**<0.05**	**>0.1**	**<0.01**	**<0.01**	**>0.05**
Significance	**-**	**-**	**-**	**n.s.**	**n.s.**	**n.s.**	**sig**	**sig**	**n.s.**		**sig**	**n.s.**

F-fasting, 1 hr-1 hour after meal , 2 hr- 2 hour after meal ,s.d.-Standard deviation ,n.t.- Not significant, sig—Significant.

Table II Laboratory investigations for group II cases.: Blood glucose estimation (mg|dl)

N^{20}	Onset		After 10 days		After 20 days		After 30 days	
	F	PP	F	PP	F	PP	F	PP
1.	156	220	150	210	148	200	140	190
2.	146	200	146	192	129	174	118	156
3.	130	200	134	200	140	198	142	204
4.	130	196	130	200	148	205	152	208
5.	154	202	140	192	130	180	120	164
6.	145	202	140	192	132	180	122	171
7.	132	198	128	192	124	184	116	170
8.	134	196	128	180	120	170	112	152
9.	136	189	130	178	124	160	116	150
10.	140	131	130	125	124	110	116	106
11.	142	181	138	176	130	174	126	170
12.	130	192	126	184	120	170	120	164
13.	132	182	126	176	120	174	118	164
14.	131	131	126	126	120	118	112	106
15.	130	185	126	174	118	170	104	150
16.	147	150	148	150	151	160	155	166
17.	128	152	132	161	132	169	142	170
18.	129	172	133	174	137	176	132	170
19.	140	212	136	200	122	180	112	164
20.	147	252	130	240	124	220	114	196
Mean	137.95	196.25	135.4	183.25	129.65	173.6	124.35	164.55
s.d.±	08.76	08.76	07.43	28.69	10.25	25.38	12.9	25.9
P -value	_	_	>0.1	>0.05	<0.01	<0.001	<0.001	<0.001
significance	_	_	n.s.	n.s.	sig	sig	sig	sig

F-fasting, 1 hr-1 hour after meal , 2 hr- 2 hour after meal , s.d.-Standard deviation ,n.s.- Not significant , sig—Significant.

Table—III Laboratory investigations for group –I cases.: Liver function tests.

10 N	**Onset**			**After 30 days**		
	S.B.	**SGPT**	**A.P.**	**S.B.**	**SGPT**	**A.P.**
1.	0.4	18	4	0.4	16	4
2.	0.6	14	5	0.5	16	3
3.	0.4	14	4	0.3	13	3
4.	0.6	12	4	0.5	15	5
5.	0.6	14	3	0.7	14	4
6.	0.2	14	5	0.3	16	6
7.	0.4	18	6	0.4	20	5
8.	0.6	19	5	0.7	20	5
9.	0.8	21	4	0.6	20	5
10.	0.4	21	4	0.5	20	4
Mean	0.5	16.5	4.4	0.49	16.7	4.4
S.D.+_	0.17	3.27	3.27	0.14	3.13	0.97
P value	-	-	-	>0.5	>0.5	>0.5
Significance	-	-	-	n.s.	n.s.	n.s.

S.B.-Serum bilirubin, SGPT-Serum glutamate pyruvate transaminase, A.P.-Alkaline phosphatase.

and twice after one hour of interval. The liver function test including Serum bilirubin ,SGPT and Alkaline phosphatase were analyzed at the start of the study and finally after 30 days of *Vinca rosea* extract administration mean SD value, p -value and significant tests were done to analysed the results.

RESULTS AND DISCUSSIONS

During this research work the gylcemic interaction of extract of *Vinca rosea* in albino mice was studied and analysed to know the effect of aqueous extract of plant on blood glucose level before and after administration as per experimental layout. Blood glucose level were estimated in fasting and post prandial states in diabetic cases after 10^{th}, 20^{th} and 30^{th} days of *Vinca rosea* extract administration. (I) Significant lowering of blood glucose levels were observed after 20^{th} and 30^{th} days recordings. The perusal of table no.-I it was observed that in group I cases the glucose lowering effect was more significant after two hours of extract treatments. The action of *Vinca rosea* in fast acting for reducing blood glucose was also observed by Chattopadhyay *et.al*.(1991)[2]. They stated that the hypoglycemic effect of *Vinca rosea* extract was at per with the tolbutamide. In table no. II the different lever function test were analyzed and recorded in table II.the results indicated in table II in group I cases indicating the effect of *Vinca rosea* extract on lever function test. It was observed that there was no significant alternation in Serum bilirubin ($p>0.1$), SGPT ($p>0.1$) and Alkaline phosphatase ($p>0.5$) even after 30^{th} days of extract treatment. It was concluded on the basis of results that the administration of *Vinca rosea* extract @ 200 mg|kg body weight has no adverse effect on liver. Alarcon-Aguilara *et al* (1998)[1] observed the effect of plant aqueous extract which help in reducing the anti- hyperglycemic effect on diabetic albino mice. Similar observations were also reported by chattopadhyaya *et al*. (1991)[2] and Grover *et al* (2002)[5]. Kaleem, *et al.* (2006)[6] observed the anti diabetic activity mediated by anti oxidant activities in plant extract, *Annona squamosa*.

CONCLUSION

Aqueous extract of *Vinca rosea* was administered in the dose of 200mg|kg body weight for one month to normal euglycemic and alloxan diabetic mice. The observations were recorded after 10^{th}, 20^{th} and 30^{th} days of the study. The effect of aqueous extract of *Vinca rosea* concluded as-

1. In euglycemic mice there was a significant reduction in blood glucose level at 20^{th} and 30^{th} day of study.
2. Since the reduction in the blood glucose level was gradually decreased by inducing release of insulin, which may be compared with tolbutamide,.
3. No toxic effect on liver was observed by the extract.

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