HYPOGLYCAEMIC ACTIVITY OF VEGETABLE FRUIT *Coccinia indica* AND ITS INTERACTION WITH GLIBENCLAMIDE IN NORMAL AND ALLOXAN INDUCED DIABETIC RATS

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Abstract: Experimentally it has been proved that the medicinal plants are valuable in controlling many diseases including hyperglycaemia in diabetics. The present paper reports the hypoglycemic effect of *Coccinia indica* and its interaction with standard hypoglycemic compound that is glibenclamide (sulfonylureas derivative) known to be useful for the treatment of diabetes in Ayurveda. Wistar rats of either sex were orally administered the fruit powder (@ 1.5 g/kg BW) for 50 days. Blood samples were withdrawn from retro-orbital plexus and were analyzed for blood glucose on 0,25and 50 days and for cholesterol, TG, BUN,ALT on 0 and 50 days .Liver glycogen was estimated on 50 days .Oral administration of fruit powder of *Coccinia indica* showed significant (P<0.01) reduction of plasma glucose level, cholesterol (P<0.01) , TG (P<0.05) and ALT (P<0.01) in alloxan induced diabetic rats at 50 day. *Coccina indica* fruit has significant hypolglycaemic activity as it lowered blood glucose level in diabetic rats. It also acts as hypolipidemic.

Key words: *Coccinia indica*, alloxan diabetics, hypoglycaemic effect, glibenclamide interaction.

Introduction: Diabetes mellitus is a chronic multifactorial metabolic syndrome due to dietetic factors and relative or absolute deficiency of insulin leading to disturbances in carbohydrate, fat and protein metabolism. The complex nature of diabetes has always posed many problems in finding out its effective treatment. Major therapeutic tool to control diabetes mellitus is insulin besides oral synthetic hypoglycaemic compounds. Treatment and management of diabetes mellitus without any side and toxic effects is still a challenge with oral synthetic hypoglycaemic compound. This leads to increasing demand for natural products with antidiabetic activity and fewer side effects. *Coccinia indica* fruit is used as fruit vegetable and regarded as useful in the treatment of diabetes like many other hypoglycaemic herbs. Present study was carried out to investigate the effect of Coccinia indica fruit powder and its interaction with oral hypoglycaemic drug i.e. glibenclamide is studied in normal and alloxan diabetic rats.

Materials and Methods: Experimental investigations were carried out to study hypoglycaemic effect of *Coccinia indica* fruit powder in normal and alloxan induced diabetic wistar rats.

Processing of plant material: Healthy, immature fruits of *Coccinia indica* were collected from agricultural farm, sliced into thin chips and shed dried under fan in the laboratory. The dried fruits were crushed into fine powder with the help of electrical grinder. The powder so obtained was used as per requirement for experimental purpose.

Experimental animals: Adult Wistar rats of either sex were procured from M/s Raj Biotech India Ltd., Wing, Satara (Maharashtra) and maintained by the Department of Veterinary Pharmacology and Toxicology were employed in the present study.Sixty wistar rats of either sex weighing between 180-220 g were randomly selected for the study. They were divided into six groups each consisting of ten rats. All the rats were housed in clean polypropylene cages in laboratory animal house and allowed to get acclimatized. They were maintained on rat feed supplied by M/s Pranav Agro Industries Ltd., Solapur (Maharashtra) with free access to fresh clean drinking water throughout the experimental period. Ethical clearance for the handling of experimental animals was obtained from the committee constituted for the purpose (CPCSEA)

Induction of diabetes: Rats were fasted overnight. Diabetes mellitus was induced by intraperitoneal administration of alloxan monohydrate (S.D.Fine Chem. Ltd., Mumbai) in physiological saline as 5% w/v (Khosla et al.,1995) at the dose rate of 120 mg/kg body weight. After one week, diabetic status was confirmed by estimating serum glucose levels. The blood samples were collected from orbital plexus. The rats showing fasting serum glucose level higher than 200 mg/dl were included in Group III, IV,V and Group VI

Administration of fruit powder: Fruit powder was administered by mixing in feed as mentioned in Table 1.the drug was administered for the period of 50 days.

Experimental design: After the induction of diabetes the rats were divided into six groups of ten animals each.

In Group V and VI diabetic rats were treated with standard hypoglycaemic compound , glibenclamide..

Blood collection and determination of blood biochemicals: Blood samples were collected from retro-orbital plexus of each rat of a Group on 0, 25 and 50 day in heparinised vials and plasma is obtained by centrifugation and glucose level is checked by GOD/POD (Glucose Oxidase Peroxidase)

end point method, cholesterol level by CHOD/POD (Cholesterol Oxidase Peroxidase) end point, triglycerides by GPO/POD (Glycerol-3-phosphate Oxidase Peroxidase) end point method, blood urea nitrogen by modified birth lot end point method and alanine transaminase (ALT) by modified IFCC kinetic method using commercial reagent kits (Ambica diagnostics)

The glycogen contents of liver were estimated by colorimetric micromethod as described by Kemp *et al*. (1954).

Statistical analysis: The biochemical parameters in all six groups were analysed by analysing the data generated by Factorial Randomised Block Design (Panse, V.G. et al.,1967). The treatment means compared by critical difference by statistical method and analysis of variance.

Results And Discussion: There was no behavioural change in all groups of rats during entire period of experimental trial. The rats in control and treatment groups had not exhibited any treatment related adverse reactions and were apparently healthy upto fifty days.

The fruit powder of *Coccinia indica* produce 10.18% (P<0.01) blood glucose reduction on 25^{th} day and 18.86% (P<0.01) on 50^{th} day in Group II, 18.37% on 25^{th} day and 26.01% (P<0.01) reduction on 50^{th} day in Group IV. In group I and III per cent reduction were 3.54% ,0.21% and 8.74% , 14.45% on 25^{th} (P<0.01) and on 50^{th} day (P<0.01) respectively. In GroupVI reduction on 25 (P<0.01) and 50 day (P<0.01) is 37.78% and 51.95% respectively. Glibenclamide produced a significant (P<0.01) reduction in blood glucose. (Table 2)

The effect of fruit powder on plasma cholesterol level in Group 1 was not significant. There was significant fall (P<0.01) in cholesterol level in Group II, IV,V,VI and significant increase (P<0.01) in Group III (Table 3).

The effect of fruit powder on plasma triglyceride level in Group I&VI was not significant .There was significant fall (P< 0.01) in Groups III,IV,V and (P<0.05) in group II (Table 2).

There was no significant difference between pre treatment and post treatment BUN concentration in Group I,II&VI.There was significant increase in (P<0.01) BUN in Group III,V& in IV (P<0.05) . (Table 4).

In Group I & II no no significant change was observed in plasma ALT levels . In Group III ALT levels were increased significantly (P< 0.01) .In Group IV significantly reduced ALT levels , in Group V & VI significantly increased (P< 0.01) ALT levels within normal range ,similar observations were reported by Dhanabal at al (2004) with toluene fraction (Table 5).

The fruit powder treatment to normal rats significantly increased (P< 0.01) the liver glycogen content. The liver glycogen content was significantly reduced (P< 0.01) in Group III, IV, V&VI. (Table 6).

There were no behavioural and treatment related adverse reactions in control and treatment group rats throughout the experimental period after oral administration of fruit powder. Vaidya *et al*. (1989) reported similar observations for acute toxicity studies of *Coccinia indica* lyophilized leaves powder which in rat and mice showed LD_{50} more than 3 g/kg and in dog 1 g/kg body weight indicating therapeutic safety

Diabetes mellitus is possibly the world largest growing metabolic disease and as the knowledge on the heterogenecity of this disorder is advanced, the need for more appropriate therapy increases (Baily et al.,1996). Traditional plant medicines are used worldwide for diabetic complications. Many author reported that different parts of *Coccinia indica* are useful in diabetes mellitus (Chandrashekhar et al.,1989, Dhanabal et al., 2004, Gupta et al., 1994). But reports of hypoglycaemic activity of fruit of *Coccinia indica* are very meagre. Due to this reason fruit powder of *Coccinia indica* was evaluated The normal control group rats did not show any significant change in plasma glucose concentrations. Normal rats treated with fruit powder showed significant fall at mid treatment and post treatment plasma glucose level. The hypoglycaemic response observed in *Coccinia indica* fruit powder in normal rats revealed possible role of pectin present in fruit powder. Diabetic control rats showed progressive increase in plasma glucose level even after fifty days. Diabetic rats treated with glibenclamide showed significant hypoglycaemic response at mid treatment and post treatment level. However, the mean per cent fall with glibenclamide treatment was higher than fruit powder treatment in alloxan diabetic rats. The concurrent administration of fruit powder and glibenclamide in diabetic rats showed significant reduction in glucose level with increase in mean per cent from mid treatment to post treatment level. The study revealed higher fall in glucose with combination of fruit powder and glibenclamide as compared to glibenclamide and fruit powder treatment alone. Normal rats treated with fruit powder showed significant rise in hepatic glycogen indicating enhanced rate of glycogensis. There was drastic reduction in liver glycogen in diabetic control. In diabetic rats treated with fruit powder hepatic glycogenic reduction was less as compared to diabetic rats treated with glibenclamide indicating enhanced rate of glycogenesis by fruit powder together showed least reduction indicating rate of glycogenesis as compared to fruit powder and glibenclamide treatment alone.The detailed studies are required to elucidate the exact mechanism of action of fruit in normal and diabetic rats. *Coccinia indica* is

commonly used fruit vegetable. Its interaction with synthetic oral hypoglycaemic compounds needs to be further investigated.

In diabetes mellitus increase in cholesterol and triglyceride is in the agreement with findings of Nikkila and Kekki (1973). Under normal circumstances insulin activates enzyme liporotein lipase and hydrolyses triglycerides. The deficiency of insulin results in failure to activate enzyme thereby causing hypertri glyceridemia. Similarly, the plasma free fatty acid concentration is elevated as a result of increased free fatty acids outflow from fat depots, where the balance of the free fatty acid esterification triglyceride lipolysis cycle is displaced in favour of lipolysis. The significant improvement of the levels of plasma cholesterol and triglyceride with fruit powder

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in diabetic rats could be directly attributed to improvements in insulin levels / utilization. Pectins from various vegetables including *Coccinia indica* which showed hypolipidemic activity mainly due to lower rate of absorption and higher rate of degradation and elimination of lipids (Kumar et al 1993).

The blood urea nitrogen levels in normal rats and in normal rats treated with fruit powder were in normal limits. In diabetic control rats BUN was significantly higher. Our study indicated a significant hypoglycaemic activity with the fruit powder of *Coccinia indica* in normal and diabetic rats. However, the detailed studies were required to elucidate the exact mechanism of action of fruit of *Coccinia indica* in normal and diabetic rat.

Table 1: Experimental groups and their treatments

Group No.	No.of rats	Treatments
I	10	Normal (control)
II	10	Normal rats fed with *Coccinia indica* Wight & Arn. fruit powder (@ 1.5 g/kg body weight)
III	10	Diabetic control rats (Alloxan monohydrate induced)
IV	10	Diabetic rats fed with *Coccinia indica* Wight & Arn. fruit powder (@ 1.5 g/kg body weight)
V	10	Diabetic rats treated with glibenclamide (@ 600 µg/kg body weight)
VI	10	Diabetic rats treated with glibenclamide (@ 600 µg/kg body weight) and *Coccinia indica* fruit powder (@ 1.5 g/kg body weight)

Table 2 : Effect of Coccinia indica fruit powder and glibenclamide on plasma glucose levels of normal and diabetic rats in various groups.

Group No.*	Plasma glucose level in mg/dl (Mean + SE)			Percent changes in plasma blood glucose	
	Pre-treatment (0 day)	Post treatment		Percent changes (Mid treatment	Percent changes (Post treatment
		Mid treatment (25 days)	Post treatment (50 days)		
I	82.84 + 1.53	79.90 + 0.99	83.02 + 1.38	3.54	0.21
II	80.38+0.94^{a}	72.19 + 1.83^{b}	65.22 + 1.53^{c}	10.18	18.86
III	238.70+3.19^{a}	259.58+4.23^{b}	273.21 + 5.02^{c}	8.74	14.45
IV	245.71 + 3.14^{a}	200.56+1.96^{b}	181.79 + 1.02^{c}	18.37	26.01
V	231.82 + 4.87^{a}	162.50+5.05^{b}	127.73 + 1.80	29.90	44.90
VI	256.72+_4.55 a	159.71^{b}±1.27	123.35+_1.79^{b}	37.78	51.95

* Each group contained ten rats.

Means bearing a,b,c, superscripts in rows are significantly different. All groups (P<0.01).

Table 3 : Effect of Coccinia indica fruit powder and glibenclamide on plasma cholesterol and triglyceride level of normal and diabetic rats in various groups.

*Group No	Plasma cholesterol level in mg/dl (mean + SE)			Plasma triglycerides level in mg/dl (Mean + SE)		
	Pre treatment (0 day)	Post treatment (50 day)	Percent changes in plasma cholest-erol	Pre treatment (0 day)	Post treatment (50 day)	Percent changes in plasma triglyce-ides
I	84.56+ 0.92	81.99 +0.82	7.60	92.25 +1.32	96.33 +1.54	4.42
II	79.95[a] + 0.96	60.91[b]+ 0.69	23.81	95.95[a] + 1.04	89.90[b]+ 1.49	6.72
III	112.78[a]+ 0.72	185.95[b]+ 0.96	64.87	140.11[a]+1.26	192.98[b]+1.07	37.59
IV	120.91[a]+0.85	139.74[b]+0.78	15.97	131.97[a]+1.00	104.97[b]+1.15	20.45
V	116.67[a]+0.72	138.34[b]+1.31	18.57	118.76[a]+2.21	143.70[b]+ 2.2	21.00
VI	125.41[a]+1.37	140.64[b]+2.72	12.14	126.58 +2.01	120.58+ 1.28	4.74

1) * Each group contained ten rats.
2) Means bearing a,b superscripts in rows are significantly different.
3) All groups show (P<0.01) except Group-II in triglycerides (P<0.05)

Table 4: Effect of Coccinia indica fruit powder and glibenclamide on plasma BUN level of normal and diabetic rats in various groups.

*Group No	Plasma BUN level in mg/dl (mean + SE)		
	Pre treatment (0 day)	Post treatment (50 day)	Percent changes in plasma BUN
I	18.60 + 0.84	19.92+0.73	7.09
II	19.39 + 0.69	19.73+0.70	1.75
III	28.13 [a] + 0.84	39.72 [b]+ 1.20	41.20
IV	30.23[a] + 0.75	32.11[b]+ 0.51	6.21
V	29.54[a] + 0.84	33.54[b] +1.20	13.54
VI	28.64 + 1.03	31.42 + 0.95	9.70

- Each group contained ten rats
- Means bearing a,b superscripts in rows are significantly different, group IV (P < 0.05) and other groups (P < 0.01).

Table 5: Effect of *Coccinia indica* Wight & Arn. fruit powder and glibenclamide on plasma ALT level of normal and diabetic rats in various groups

Group No.*	**Treatment**	**Plasma ALT level in IU/l (mean + SE)**		**Per cent changes in plasma ALT levels**
		Pre-treatment (0 day)	**Post-treatment (50 day)**	
I	Normal (control)	42.50 +1.23	43.51 +0.87	2.37
II	Normal + *Coccinia indica* Wight & Arn.@ 1.5 g/kg body weight	46.17 +1.00	47.77 +0.76	3.46
III	Diabetic control	50.26[a] +0.81	79.58[b] +1.46	58.33
IV	Alloxan diabetic + *Coccinia indica* Wight & Arn. @ 1.5 g/kg body weight	61.33[a] +1.05	46.99[b] +1.43	23.38
V	Alloxan diabetic + glibenclamide @ 600 µg/kg body weight	51.71[a] +0.97	64.11[b] +0.93	23.97
VI	Alloxan diabetic + glibenclamide @ 600 µg/kg body weight + *Coccinia indica* Wight & Arn. @ 1.5 g/kg body weight	55.27[a] +0.98	69.85[b] +1.04	26.37

* Each group contained ten rats.
Means bearing a, b superscripts in rows are significantly different (P<0.01)

Table 6: Effect of *Coccinia indica* fruit powder on liver glycogen level in normal and diabetic rats in various groups.

*Group No	Liver glycogen in g% wet weight of tissue	Per cent increase (+) or decrease (-)
I	2.10^{a} + 0.015	-
II	2.24^{b} + 0.010	(+) 6.66
III	1.13^{c} + 0.010	(-) 46.19
IV	1.81^{c} + 0.09	(-) 13.80
V	1.74^{c} + 0.010	(-) 17.14
VI	1.98^{c} + 0.002	(-) 5.71

Each group contained ten rats

- Means bearing common superscripts are significantly lower ($P < 0.01$) when compared to normal control.
- Means bearing b superscripts is significantly higher ($P < 0.01$) when compared to normal control.

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