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EFFECT OF VITAMIN C ON BLOOD GLUCOSE LEVEL IN RABBITS AND ITS POSSIBLE INTERACTION WITH COMMONLY USED ANTI DIABETIC AGENTS

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ABSTRACT:

Effect of Vit. C on glucose and its possible interactions with oral antidiabetic agents (i.e. glimepiride, gliclazide and metformin) in rabbits are reported within the present study. The study was performed on albino rabbits within which hyperglycemia was induced by giving glucose. We observed that Vit.C significantly lowered the glucose level but on co-administration with oral antidiabetic agents it antagonized the effect of the latter drugs. It is therefore advisable to not give excess amount of vit.C to diabetic patients just in expectation that antioxidants are helpful to diabetics and don't produce adverse effects.

KEYWORDS: Glucose, Vit. C, antioxidants, glimepiride, gliclazide, metformin.

INTRODUCTION

Diabetes Mellitus is a disease known to humans since ages. In Indian literatures like Caraka Samhita descriptions of polyuric conditions resembling diabetes is present comprising of Polyuria and sweet urine.

Etiology of Diabetes is multifactorial and is characterised by disturbed carbohydrate, protein and lipid metabolism. The fundamental defect is in secretion of the hormone Insulin or its action at the tissue level.

Chronic hyperglycemia have profound effects on most systems of the body. The toxic effects of hyperglycemia may result from accumulation of glycosylated products[1], increased sorbitol, formation of diacylglycerol resulting in activation of protein kinase C or free radical generation.[2]

Free radicals especially superoxide may have role in destruction of b cell in type 1 diabetes.[3] Therefore antioxidants may have role in retarding or preventing the process of diabetes by reducing the formation of free radicals.[4,5,6,7]

Therapy of Diabetes requires a continued supervision for optimal blood glucose level in patients because strict glucose level can prevent the complications of diabetes namely retinopathy, nephropathy, neuropathy and vasculopathy. At the other end avoidance of hypoglycemia is additionally important because that may cause convulsions, coma and death.

Therefore it becomes necessary to understand interactions of varied pharmacological agents with antidiabetic agents because knowledge of such an interaction can avoid or mitigate such an interaction by adjustment of doses and schedule of drug administration .

Various drugs have known interaction with antidiabetic agents, some causing hypoglycemia, some hyperglycemia and altering the response of diabetic patients to their existing therapeutic regimens. [8,9]

Salicylates, , ethanol, sulfonamides and pentamidine enhances the hypoglycemic effect while glucocorticoids, thiazides , diazoxide, minoxidil , heparin and nicotine antagonizes the effect of antidiabetic agents.

It was therefore decided to to explore the chances of drug interactions between antioxidants and antidiabetic agents.

The aim of this study is -

1) To assess if Vit. C itself had any effect on glucose level.
2) To judge the possible effect of Vit C on the changes of blood glucose levels produced by antidiabetic agents.

The various drugs included within the study are those which are commonly employed e.g. Vitamin C, gliclazide, glimepiride and metformin).

MATERIAL AND METHODS

Present study was conducted on healthy albino rabbits of either sex weighing 1.5 - 2.0 kg. The animals were made available within the animal house of Department of Pharmacology. They were maintained on gram diet.

Estimation of glucose level was done by using glucose oxidase - peroxidase method because that is the most accurate and specific method

For this study rabbits were divided into 8 groups of 5 rabbits each.

Group I : Rabbits of this group got 25 gm of glucose powder orally. This group shows normal pattern of blood glucose level after a glucose load and served as control.

Group II : Rabbits of this group got vit C (100 mg/kg/d) orally for 7 days and 25 gm of glucose powder orally on 7^{th} day. This group shows effect of vitamin C on blood glucose level.

Group III : Rabbits of this group got glimepiride (0.2 mg/kg) orally and 25 gm of glucose powder. This group shows normal pattern of antidiabetic action of glimepiride.

Group IV :: Rabbits of this group were on vitamin C (100 mg/d) for 7 days and glimepiride (0.2 mg/kg) plus 25 gm of glucose powder were added on 7th day. This group shows effect of vitamin C on the pattern of blood glucose levels produced by glimepiride.
Group V : Rabbits of this group got gliclazide (8 mg/kg) orally and 25 gm of glucose powder. This group shows normal pattern of antidiabetic action of gliclazide.
Group VI : Rabbits of this group were on vitamin C (100 mg/kg/d) for 7 days and gliclazide (8 mg/kg) plus 25 gm of glucose powder were added on 7th day. This group shows effect of vitamin C on the pattern of blood glucose levels produced by gliclazide.
Group VII : Rabbits of this group got metformin (100 mg/kg) orally and 25 gm of glucose powder. This group shows normal pattern of antidiabetic action of metformin.
GroupVIII : Rabbits of this group were on vitamin C (100 mg/ Kg/d) for 7 days and metformin (100 mg/kg) plus 25 gm of glucose powder were added on 7th day. This group shows effect of vitamin C on the pattern of blood glucose levels produced by metformin.

- Rabbits were fasted overnight and during the study but were allowed water during this period.
- Samples were collected just before administration of drug and glucose (0 hr.) and after 2, 4 and 6 hrs. of administration.
- All drugs and glucose were given orally through nasogastric tubes, those not soluble in water were rendered soluble with the help of gum acacia.

OBSERVATIONS

TABLE - 1

Blood glucose levels of rabbits receiving 25 gm glucose powder after overnight fasting.

S.No.	Blood glucose level (mg%) Time interval (hrs.)			
	0	2	4	6
1.	104.2	297.4	129.2	112.7
2.	111.0	288.8	133.1	108.5
3.	103.7	291.3	126.9	99.8
4.	98.9	302.0	119.3	118.0
5.	106.0	293.5	117.2	97.8
Mean	104.62	294.6	125.14	107.36
S.E. +	1.98	2.33	3.0	3.82

TABLE - 2

Blood glucose levels of rabbits receiving Vitamin C (100 mg./kg/d) for 7 days and 25 gm glucose powder after overnight fasting.

S.No.	Blood glucose level (mg%) Time interval (hrs.)			
	0	2	4	6
1.	98.5	276.9	84.2	84.6
2.	102.5	271.0	67.8	69.0
3.	94.8	312.0	89.4	84.2
4.	89.3	286.3	92.1	71.4
5.	92.0	263.0	97.4	89.4
Mean	95.42	281.84	86.18	79.72
S.E. +	2.34	8.46	5.07	4.02

TABLE - 3

Blood glucose levels of rabbits receiving glimepiride (0.2 mg/kg) and 25 gm glucose powder after overnight fasting.

S.No.	Blood glucose level (mg%) Time interval (hrs.)			
	0	2	4	6
1.	104.2	112.0	101.8	98.3
2.	108.0	116.3	104.5	102.7
3.	112.9	127.1	98.3	104.9
4.	99.3	110.4	109.7	93.6
5.	102.0	109.8	112.0	90.5
Mean	105.28	114.96	105.26	98.00
S.E. +	2.38	3.28	2.51	2.70

TABLE - 4

Blood glucose levels of rabbits receiving Vitamin C (100 mg/kg/d) for 7 days and glimepiride (0.2 mg / kg) plus 25 gm glucose powder after overnight fasting.

S.No.	Blood glucose level (mg%) Time interval (hrs.)			
	0	2	4	6
1.	109.0	163.6	112.1	108.0
2.	115.0	193.9	78.9	69.6
3.	96.9	178.7	115.1	72.7
4.	103.0	160.6	118.1	63.6
5.	112.1	166.6	133.3	81.2
Mean	107.2	172.68	111.46	79.02
S.E. +	3.26	6.14	8.99	7.80

TABLE - 5

Blood glucose levels of rabbits receiving gliclazide (8 mg/kg) and 25 gm glucose powder after overnight fasting.

S.No.	Blood glucose level (mg%) Time interval (hrs.)			
	0	2	4	6
1.	107.2	101.7	79.4	81.3
2.	100.0	91.4	76.0	90.3
3.	94.9	86.3	73.7	84.8
4.	98.8	88.0	81.2	91.7
5.	92.7	107.0	92.0	79.6
Mean	98.72	94.88	80.46	85.54
S.E. +	2.50	4.05	3.17	2.39

TABLE - 6

Blood glucose levels of rabbits receiving Vitamin C (100 mg/kg/d) for 7 days and gliclazide (8 mg / kg) plus 25 gm glucose powder after overnight fasting.

S.No.	Blood glucose level (mg%) Time interval (hrs.)			
	0	2	4	6
1.	87.2	140.0	78.3	79.0
2.	91.4	128.5	102.1	83.2
3.	84.0	177.1	99.0	88.3
4.	89.8	151.4	93.0	90.0
5.	97.0	162.8	121.5	93.6
Mean	89.88	151.96	98.78	86.82
S.E. +	2.18	8.51	7.01	2.58

TABLE - 7

Blood glucose levels of rabbits receiving metformin (100 mg/kg) and 25 gm glucose powder after overnight fasting.

S.No.	Blood glucose level (mg%) Time interval (hrs.)			
	0	2	4	6
1.	112.0	197.8	128.1	98.7
2.	108.3	186.4	118.7	101.3
3.	104.7	201.7	131.3	100.0
4.	113.9	211.9	124.8	103.8
5.	118.1	201.0	129.4	112.0
Mean	111.4	199.76	126.46	103.16
S.E. +	2.30	4.10	2.21	2.37

TABLE - 8

Blood glucose levels of rabbits receiving Vitamin C (100 mg/kg/d) for 7 days and metformin (100 mg / kg) plus 25 gm glucose powder after overnight fasting.

S.No.	Blood glucose level (mg%) Time interval (hrs.)			
	0	2	4	6
1.	93.9	193.0	108.0	119.7
2.	97.0	197.8	121.7	126.3
3.	90.9	186.4	134.5	130.0
4.	88.4	149.0	117.60	109.8
5.	82.7	181.0	123.0	112.0
Mean	90.58	181.44	120.96	119.56
S.E. +	2.44	8.62	4.29	3.92

TABLE - 9

Statistical comparison between the group receiving Vit. C plus glucose powder and control group (receiving only glucose powder)

Group receiving Vit. E plus glucose powder (n = 5)	Time interval (hrs.)			
	0	2	4	6
Mean blood glucose (mg%)	95.42	281.84	86.18	79.72
SD +	5.23	18.92	11.34	8.99

Control group (receiving glucose powder only) (n = 5)	Time interval (hrs.)			
	0	2	4	6
Mean blood glucose (mg%)	104.62	294.6	125.14	107.36
SD +	4.43	5.21	6.71	8.54
't' value	3.0	1.45	6.61	4.98
'p' value	< 0.05	> 0.05	< 0.01	< 0.01

TABLE - 10

Statistical comparison between the group receiving glimepiride plus Vit. C plus glucose powder and the group receiving glimepiride and glucose powder

Group receiving glimepiride, Vit. C and glucose powder (n = 5)	Time interval (hrs.)			
	0	2	4	6
Mean blood glucose (mg%)	107.2	172.68	111.46	79.02
SD +	7.30	13.75	20.13	17.47

Group receiving glimepiride and glucose powder (n = 5)	Time interval (hrs.)			
	0	2	4	6
Mean blood glucose (mg%)	105.28	114.96	105.26	98.0
SD +	5.32	7.33	5.61	6.04

't' value	0.47	8.29	0.66	2.29
'p' value	> 0.05	< 0.001	> 0.05	> 0.05

TABLE - 11

Statistical comparison between the group receiving gliclazide plus Vit. C plus glucose powder and the group receiving gliclazide and glucose powder

Group receiving gliclazide, Vit. C and glucose powder (n = 5)	Time interval (hrs.)			
	0	2	4	6
Mean blood glucose (mg%)	89.88	151.96	98.78	86.82
SD +	4.88	19.06	15.70	5.77

Group receiving gliclazide and glucose powder (n = 5)	Time interval (hrs.)			
	0	2	4	6
Mean blood glucose (mg%)	98.72	94.88	80.46	85.54
SD +	5.59	9.06	7.09	5.34
't' value	2.67	6.05	2.37	0.22
'p' value	< 0.05	< 0.01	> 0.05	> 0.05

TABLE - 12

Statistical comparison between the group receiving metformin plus Vit. C plus glucose powder and the group receiving metformin and glucose powder

Group receiving metformin, Vit. C and glucose powder (n = 5)	Time interval (hrs.)			
	0	2	4	6
Mean blood glucose (mg%)	90.58	181.44	120.96	119.56
SD +	5.46	19.30	9.60	8.78
Group receiving metformin and glucose powder (n = 5)	Time interval (hrs.)			
	0	2	4	6
Mean blood glucose (mg%)	111.4	199.76	126.46	103.16
SD +	5.14	9.17	4.94	5.30
't' value	7.11	1.91	1.14	3.58
'p' value	< 0.001	> 0.05	> 0.05	< 0.05

DISCUSSION

The aim of this study was to ascertain the effect of vit C on blood sugar level and its possible interactions with oral antidiabetic agents. Present study was conducted on healthy albino rabbits of either sex weighing 1.5 - 2 Kg.

There were 8 groups within the present study, each group comprising of 5 rabbits. The rabbits were fasted overnight. Blood samples (0.5 ml) were withdrawn by disposable syringe every time from marginal pinna vein. Samples were taken in fluoride vials. Samples were centrifuged for 10 minutes at 3000 r.p.m. Supernatant was taken for estimation of glucose . The estimation was done by glucose oxidase - peroxidase method.

The observations obtained are shown in tables.

Table 1 shows glucose levels in fasted rabbits and at 2, 4 and 6 hrs following administration of 25 gm glucose powder orally. The mean of glucose levels at '0' hour (just before glucose powder administration) i.e. fasting value was found to be 104.62 mg%. The fasting values were found to be raised i.e. 294.6 mg% after 2 hrs, 125.14 mg% after 4 hrs and 107.36 mg% after 6 hrs following administration of glucose. These observations indicated that the glucose levels were significantly raised ($p< 0.001$) (about 3 times higher) than the fasting values following administration of a larger dosage of glucose after 2 hrs. The values declined gradually reaching almost to fasting values after 6 hrs. This group served as control for the study.

Table 2 shows glucose levels of fasted rabbits following administrate of vitamin C and glucose powder. Vitamin C was given in dose of 100 mg/kg/d for 7 days regularly and 25 glucose powder was added on 7th day and blood samples were taken. The mean of blood glucose levels at '0' hr (just before administration of drug and glucose) was 95.42 mg% which became 281.84 mg% after 2 hrs, 86.18 mg% after 4 hrs and 79.72 mg% after 6 hrs following administration of drug and glucose. These values were compared with control group in Table 9. Vitamin C was found to decrease the blood glucose level as compared to control group. The effect wasn't significant at 2 hr ($p > 0.05$) but highly significant after 4 hrs and 6 hrs ($p< 0.01$) of administration of drug and glucose.

Table 3 shows glucose level of fasted rabbits following administration of 25 gm of glucose powder and glimepiride (0.2 mg/kg) orally. The mean of blood glucose levels at '0' hr. (fasting) was 105.28 mg%, which became 114.96 mg% after 2 hrs, 105.26 mg% after 4 hrs and 98.0 mg% after 6 hrs following administration of drug and glucose. As compared to Table 1 it had been found that glimepiride exhibit significant hypoglycemia at 2 and 4 hrs.

Table 4 shows glucose level in fasted rabbits following administration of vitamin C, glucose and glimepiride. Vitamin C was given in dose of 100 mg/kg/d regularly for 7 days orally, glucose powder 25 gm and glimepiride (0.2 mg/kg) were added on 7th day and blood samples were taken. The mean of glucose levels at '0' hr (fasting) was 107.2 mg% which became 172.68 mg% at 2 hrs, 111.46 mg% at 4 hrs and 79.02 mg% at 6 hrs following administration of drugs and glucose.

The values of Table 4 were compared with those of Table 3 in Table 10. The effect of glimepiride was found to be modified in combination with vitamin C. This shows that vitamin C antagonized the effect of glimepiride at 2 hrs. This effect was highly significant ($p<0.001$).

Table 5 shows glucose levels of fasted rabbits following administration of glucose powder (25 gm) and gliclazide (8 mg/kg). The mean of glucose levels at '0' hr (fasting) was 98.72 mg% which became 94.88 mg% at 2 hrs, 80.46 mg% at 4 hrs and 85.54 mg% at 6 hrs following administration of drugs and glucose. Gliclazide produced significant hypoglycemia at 2, 4 and 6 hrs.

Table 6 shows glucose levels following administration of vitamin C, glucose and gliclazide in fasted rabbits. Vitamin C was given in dose of 100mg/kg/d for 7 days regularly, glucose powder (25 gm) and gliclazide (8 mg/kg) were added on 7th day and blood samples were taken. At '0' hr (just before administration of drug and glucose) i.e. fasting mean of glucose level was 89.88 mg% which became 151.96 mg% after 2 hrs, 98.78 mg% after 4 hrs and 86.82 mg% after 6 hrs following administration of drugs and glucose.

These values were compared with those of Table 5 in Table 11. It can be seen that vitamin C when co-administered with gliclazide, antagonized the effect of gliclazide significantly at 2 hrs ($p<0.01$).

Table 7 shows glucose levels following administration of glucose powder (25 gm) and metformin (100mg/kg) in fasted rabbits. The mean of glucose levels at '0' hr (just before administration of drug and glucose) was 111.4 mg% which becomes 199.76 mg% after 2 hrs, 126.46 mg% after 4 hrs and 103.16 mg% after 6 hrs. Metformin produced significant hypoglycemia at 2 hrs as compared to control group.

Table 8 shows glucose levels following administration of vitamin C, glucose and metformin. Vitamin C was given in dose of 100 mg/kg/d orally for 7 days regularly, glucose powder (25 gm) and metformin (100 mg/kg) were added on 7th day and blood samples were taken. The mean of glucose levels at '0' hr (just before administration of drug and glucose) was 90.58 mg% which becomes 181.44 mg% at 2 hrs, 120.96 mg% at 4 hrs and 119.56 mg% at 6 hrs following administration of drugs and glucose.

These values were compared with those of Table 7 in Table 12. Effect of metformin was found to be significantly decreased ($p<0.05$) by vit. C at 6 hrs.

CONCLUSION:

In studied conditions vit C produced significant antihyperglycemic effect at 4 and 6 hrs in experimental rabbits.

Administration of vit. C with glimepiride and glucose exhibited antagonistic effects on blood glucose levels. However these effects in studied conditions were found to be significant at 2 hrs only.

Vitamin C on co-administration with gliclazide in studied conditions had produced significant antagonistic effects at 2 hrs.

The effect of vit. C within similar conditions was noted to decrease the action of metformin significantly at 6 hrs.

Thus it's evident from this study that antioxidants e.g. Vit. C in spite of its inherent vital properties have some inevitable effects on the action of oral antidiabetic agents.

These antioxidants may moderate the effects of the drugs (in co-ordination therapy). Therefore it is advisable to prescribe and use the oral antidiabetic agents carefully with utmost

precautions in patients affected by diabetes mellitus to avoid the adverse interactions in with antioxidants.

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