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Study of Long Term Effect of Voglibose on Fasting Blood Sugar and Glycosylated Hb in Alloxan Induced Diabetic Rabbits.

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ABSTRACT

Voglibose has been clinically used as a drug which improves glucose tolerance by inhibiting digestion and absorption of glucose from intestine. Another advantage of voglibose is that it decreases post prandial glucose without inducing hypersecretion of insulin. In this study we evaluated the effects of long term voglibose treatment on changes in fasting blood glucose level together with changes in glycosylated Hb level. Study the effect of voglibose on FBS and glycosylated Hb in alloxan induced diabetic rabbits. The rabbits were divided into six experimental groups. All 36 rabbits were induced diabetic by administering alloxan IV in the marginal ear vein of rabbits. Rabbits exhibiting fasting blood glucose more than 150 gm/dl after a stabilization period of seven days were considered as diabetic. Rabbits were randomly divided into six groups. Rabbits of group I received 5ml of normal saline as placebo daily and served as control. Rabbits of group II, group III and group IV were treated with voglibose 0.25, 0.5 mg/kg and 1 mg/kg. Rabbits of group V were treated with metformin 50mg/kg and group VI were treated with combination of 50 mg/kg and voglibose1mg/kg body weight daily by gavage method for 24 weeks . FBS and PPBS at 1st hour and 2nd hour were done with help of glucometer daily for throughout 24 weeks. There was a significant decrease in FBS and HbA1c in groups treated with voglibose. Treatment with voglibose decreases insulin secretion indirectly through delaying in intestinal absorption of carbohydrate. Voglibose at different doses significantly decreased FBS and HbA1c. By decreasing insulin secretion it indirectly prevents the development of coronary artery disease which is more likely to develop in patients with impaired glucose tolerance. The effect of long term treatment of voglibose is that it can prevent the progression of impaired glucose tolerance to Type II DM.

Keywords: Alloxan, voglibose, Metformin, Diabetes, FBS, glycosylated Hb

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INTRODUCTION

Diabetes mellitus is a major global health problem and an increasing cause of morbidity and mortality. The term diabetes mellitus describes a metabolic disorder of multiple aetiology, characterized by chronic hyperglycemia with disturbance of carbohydrate,fat and protein metabolism [1]. Presently DM is an incurable metabolic disorder which affects about 2.8% of global population. The prevalence is predicted to be double by 2025. Type–ll DM is more prevalent than type-l DM. Type-ll DM is a progressive disease resulting from either defect in insulin secretion, insulin action or both [2]. In recent years many of the metabolic impairments associated with diabetes mellitus have been traced to defects in insulin action [3]. Indeed, dysfunction of glucose metabolism is closely related to defects in insulin secretion. The plasma glucose level is abnormally elevated as a result of glucose metabolism [4]. The abnormal increase in blood glucose plays a pathogenic role in metabolic disorders [5,6]. Post prandial hyperglycemia otherwise known as Impaired Glucose Tolerance stage (IGT), is a stage of impaired glucose regulation that is present in individuals whose glucose tolerance is above the conventional normal range but lower than the level considered diagnostic of type-ll diabetes mellitus . IGT represents transient stage between normal glucose tolerance and type-ll DM. Post prandial hyperglycemia or IGT plays a central role in development and progression of diabetic complications particularly cardiovascular diseases. Temelkova, Kurktschiev et al. reported that post prandial hyperglycemia and glycemic spikes were more strongly associated with atherosclerosis than fasting glucose or HbA1c [7]. Several new pharmacological agents have been developed to focus on post prandial glucose control. These include rapid acting insulin analogues [8], non sulphonylurea rapid acting insulin secretagogues [9,10], and digestive enzyme inhibitors [11,12]. Alfa-glucosidase inhibitors act as competitive inhibitors of internal alfa-glucosidase. Alfa-glucosidase is an enzyme present in the intestinal brush border which is responsible for digestion of oligosaccharide like maltose, maltriose and dextrins and produces monosaccharide like glucose, galactose and fructose which are rapidly absorbed across the wall of small intestine It has been reported that alfa-glucosidase inhibitors like acarbose, voglibose and miglitol inhibits alfa-glucosidase and reduces post prandial hyperglycemia and thereby improves glucose tolerance [13-15]. In UKPDS Study [16] and patients receiving alfa-glucosidase inhibitor acarbose [17] had lowered HbA1c over 3 years compared with placebo. Acarbose is the first alfa-glucosidase inhibitor for the treatment of post postprandial hyperglycemia. It showed significant improvement in glycemic control in type-ll DM patients. Voglibose a new alfa-glucosidase inhibitor is reported to be 20-30 times more potent than acarbose in inhibiting small intestine disaccharidases [18]. Beneficial effect of voglibose has already been demonstrated both in humans and rodents with diabetes [19].

Voglibose has been clinically used as a drug which improves glucose tolerance by inhibiting digestion and absorption of glucose from intestine. Another reported advantage of voglibose is that it decreases post prandial glucose without inducing hyper secretion of insulin [20,21]. The effect is desirable in treatment of NIDDM patients for at least two reasons i.e. hyper insulinemia may be related to the development of coronary artery disease [22,23] and hyper secretion of insulin may result in exhaustion of pancreatic beta cells [24,25]. The decrease in post prandial plasma glucose may result indecrease in the intrinsic insulin secretion

and may inhibit the over work of the pancreatic beta cell. However the long term effects of voglibose treatment on fasting blood glucose and glycosylatedHb have not been investigated. In this study we evaluated the effects of long term vogligose treatment on changes in fasting blood glucose level together with changes in glycosylated Hb level.

Objectives

To evaluate the beneficial effect of voglibose(alfa-glucosidase inhibitor) on fasting glucose and glycosylated Hb in alloxan induced diabetic rabbits.

MATERIALS AND METHODS

Thirty six healthy rabbits (New Zealand white) weighing between 1000 to 1800 gm were housed at Central Animal House of IMS and SUM Hospital Bhubaneswar. The study protocol was approved by IAEC (S 'O' A UNIVERSITY, Bhubaneswar) July 2013. The animal were kept in cages under standard laboratory condition (light period 8.00 am to 8.00 pm ,and at temp 20-24^{o}C), relative humidity 55%,fodder and water available ad libitum) . The animals received animal care [26].

The rabbits were divided into six experimental groups. All 36 rabbits were induced diabetic by administering alloxan IV in the marginal ear vein of rabbits [27-29]. Doses of alloxan required to induce diabetes were according to key body weight of the rabbits [27,30]. Rabbits exhibiting fasting blood glucose more than 150 gm/dl after a stabilization period of seven days were considered as diabetic [31]. Rabbits were randomly divided into six groups. Rabbits of group I received 5ml of normal saline as placebo daily and served as control. Rabbits of group II, group III and group IV were treated with voglibose 0.25, 0.5 mg/kg and 1 mg/kg. Rabbits of group V were treated with metformin 50mg/kg and group VI were treated with combination of 50 mg/kg and voglibose body weight daily by gavage method for 24 weeks . FBS and PPBS at 1st hour and 2nd hour were done with help of glucometer (ONE TOUCH, select one, life scan) daily for 24 weeks. Blood samples were collected from marginal ear vein only thrice throughout 24 weeks: once before induction, on 8th day after induction and on 24th weeks of experiment for estimation of glycosylated Hb.

RESULTS AND DISCUSSION

Observations were subjected to explorative data analysis to study the nature and pattern of distribution. Fasting blood sugar level could not confirm to test of normality for week 12 and week 20. Box plot at fig.1.presented comparative distribution of FBS of different treatment groups at different time periods like day 1,day21,week12 and week 20. This indicated a downward shift in FBS over the time in all the groups with varying degree but voglibose (1mg/kg) with metformin (50mg/kg) has additional advantage in lowering down the FBS level over others.There are some extreme values and outliars in the data as evident from the Box plot in (figure 1). Since data did not confirm to test of normality at certain time period and there is presence of extreme values and outliars,non parametric test like Kruskal Wallis test

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was used along with descriptive analysis (Table 1). presented descriptivestatistics of FBS fordifferent time period of study along with graphical analysis at Figure2. It is revealed that mean FBS level was maintained at more or less the same level with slight decrease during the 16week and 20 week period for the placebo group. In other five groups there was distinct fall in FBS over the time and fall was sharper in group IV(vog1mg/kg),V(met 50mg/kg)and VI (combination of vog 1mg/kg and met 50mg/kg) but sharpest for group VI (i.e. combination of vog 1mg/kg and metformin 50 mg/kg).

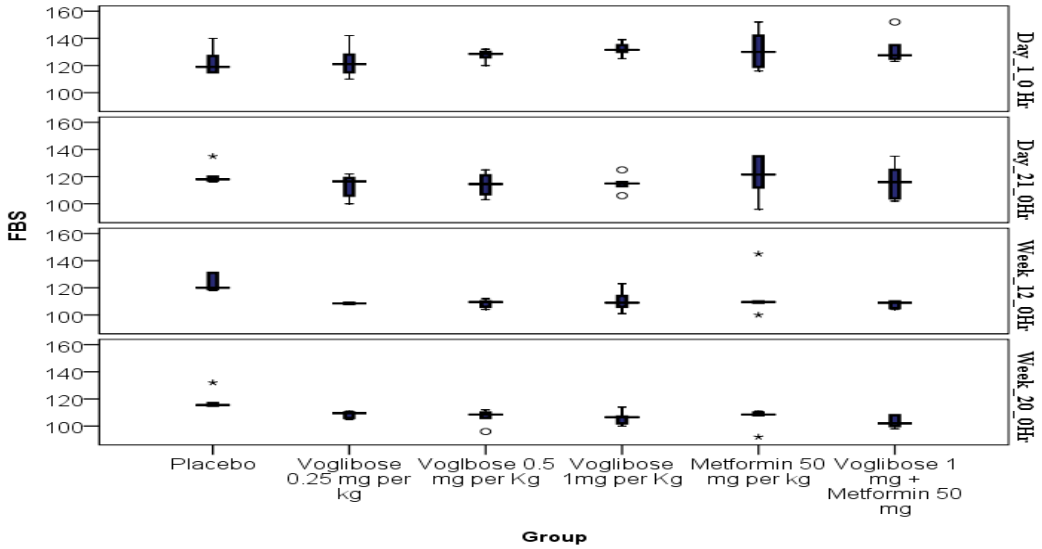

Figure 1: Comparative distribution of FBS of Different Treatment Groups(Box Plot).

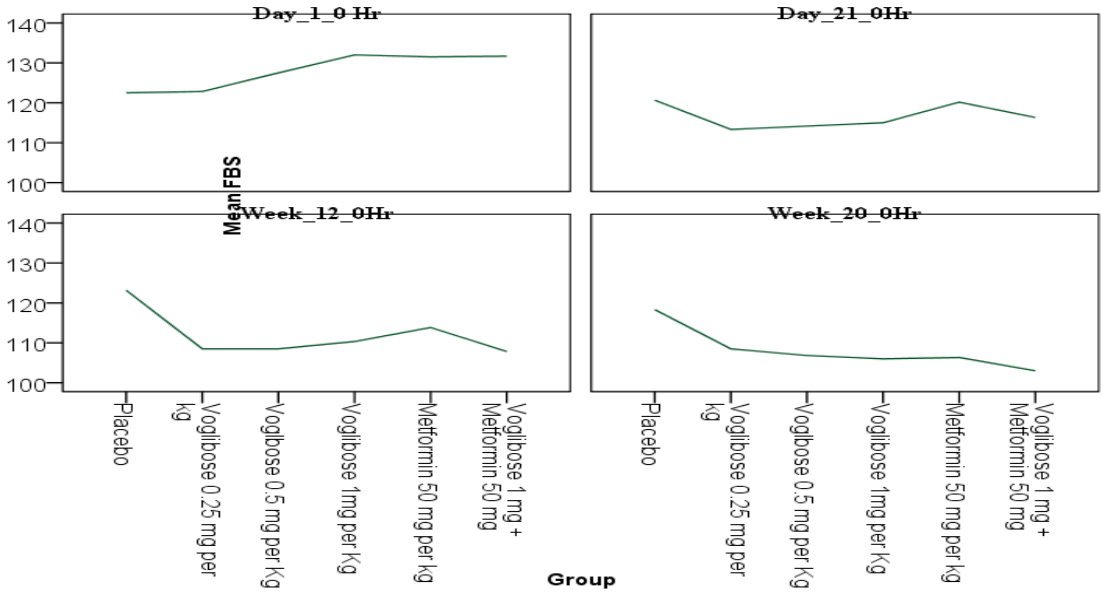

Figure 2: Trend of Fasting Blood Sugar in different Treatment Groups.

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Table 1: Descriptive Statistics of Fasting Blood Sugar for different time period of Study.

Time Period	Placebo	Voglibose 0.25 mg per kg	Voglibose 0.5 mg per Kg	Voglibose 1mg per Kg	Metformin 50 mg per kg	Voglibose 1 mg + Metformin 50 mg
	Mean FBS +-SE	Mean FBS +-SE	Mean FBS +-SE	Mean FBS +-SE	Mean FBS +-SE	Mean FBS +-SE
Day_1_0 Hr	122.5 ± 4.006	122.833±4.600	127.500±1.746	132.000±1.932	131.500±5.720	131.667±4.440
Day_7_0 Hr	122.833 ± 2.651	120.500±3.722	124.833±2.056	121.167±2.469	124.833±6.096	122.667±4.897
Day_14_0Hr	120.833±6.494	119.667±3.159	119.333±3.451	119.500±3.748	121.500±7.164	116.167±6.740
Day_21_0Hr	120.667±2.929	113.333±3.480	114.167±3.563	115.000±2.490	120.167±6.426	116.333±5.207
Week_4_0Hr	124.000±3.992	115.333±2.679	113.167±3.544	116.667±2.333	118.167±3.825	115.167±3.877
Week_6_0Hr	123.667±3.621	113.167±2.561	112.500±2.837	113.000±1.932	114.833±5.016	111.333±2.704
Week_8_0Hr	122.167±2.725	109.667±1.745	114.167±3.016	114.333±1.520	117.167±4.833	110.833±4.269
Week_10_0Hr	123.500±3.510	111.667±1.820	112.667±2.011	115.167±2.272	111.833±3.439	110.333±3.273
Week_12_0Hr	123.167±2.496	108.500±0.224	108.500±1.204	110.333±3.073	113.833±6.426	107.833±1.078
Week_14_0Hr	122.833±2.626	108.333±1.054	107.000±1.751	111.833±2.386	111.333±2.985	104.833±1.195
Week_16_0Hr	120.333±3.252	107.500±0.847	106.500±1.176	108.333±1.892	109.167±2.574	105.667±1.308
Week_18_0Hr	119.667±2.290	108.333±0.558	108.167±2.286	108.667±2.028	107.333±2.765	105.333±2.171
Week_20_0Hr	118.333±2.753	108.500±0.992	106.833±2.315	106.000±1.983	106.333±2.906	103.000±1.693

Table 2: Kruskal Wallis Test for test of significance of treatment effect.

Group	*Mean Rank Fasting Blood Sugar*			
	Day_1_0 Hour	*Day_21_0 Hour*	*Week_12_0 Hour*	*Week_20_0 Hour*
Placebo	12.00	23.25	31.83	33.50
Voglibose 0.25 mg per kg	12.75	15.92	12.25	19.58
Voglbose 0.5 mg per Kg	19.33	16.83	16.58	17.42
Voglibose 1mg per Kg	24.75	16.08	16.75	13.25
Metformin 50 mg per kg	20.92	20.83	19.17	18.00
Voglibose 1 mg + Metformin 50 mg	21.25	18.08	14.42	9.25
Significant "p" Value	***0.222***	***0.798***	***0.020***	***0.002***

In Group V the SE of means at different time period were distinctly higher than the groups with placebo. In Group VI where we administer metformin with voglibose 1mg/kg the SE even though lower than the metformin alone was higher than the groups with voglibose alone this indicated that metformin alone and met plus vog group exhibited better impact in reducing the FBS but with more variation in comparison to voglibose .25mg,.5mg and 1 mg groups.

Kruskalwallis test for test of significance of treatment effect is presented in table 2.for four time period day 1,day 21,week 12 and week 20. In day 1 and day 21 there was no significant difference in treatment effect among groups with p=0.222 and p=0.798 respectively. In week 12 and week 20 revealed significant difference among the groups p=0.20 and p=0.002 respectively.

Glycosylated Hb was compared for six groups for three time period i.e before treatment, after induction and 20 weeks after treatment.Comparison of distribution of GHb has been attempted with the help of Box plot (figure.3). The pattern is that in all the groups the median level of Hb along with Quartiles sharply increased after induction. In the placebo group there is

slight fall after 20 weeks but in voglibose with different doses there is good fall in median value before treatment. But in vog1mg with metformin, it has almost comedown to the pre induction period.

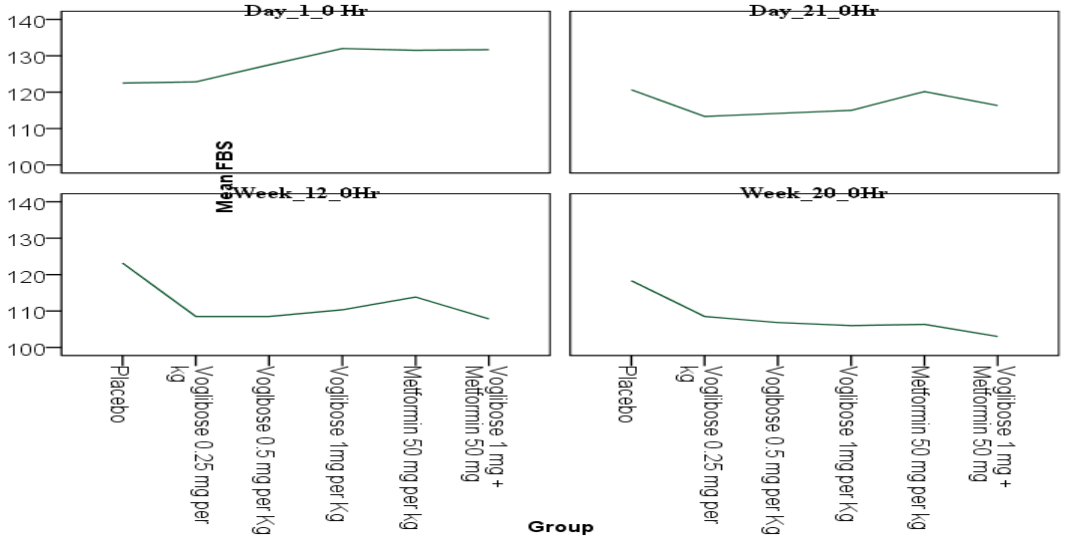

Figure 3: Trend of Fasting Blood Sugar in different Treatment Groups.

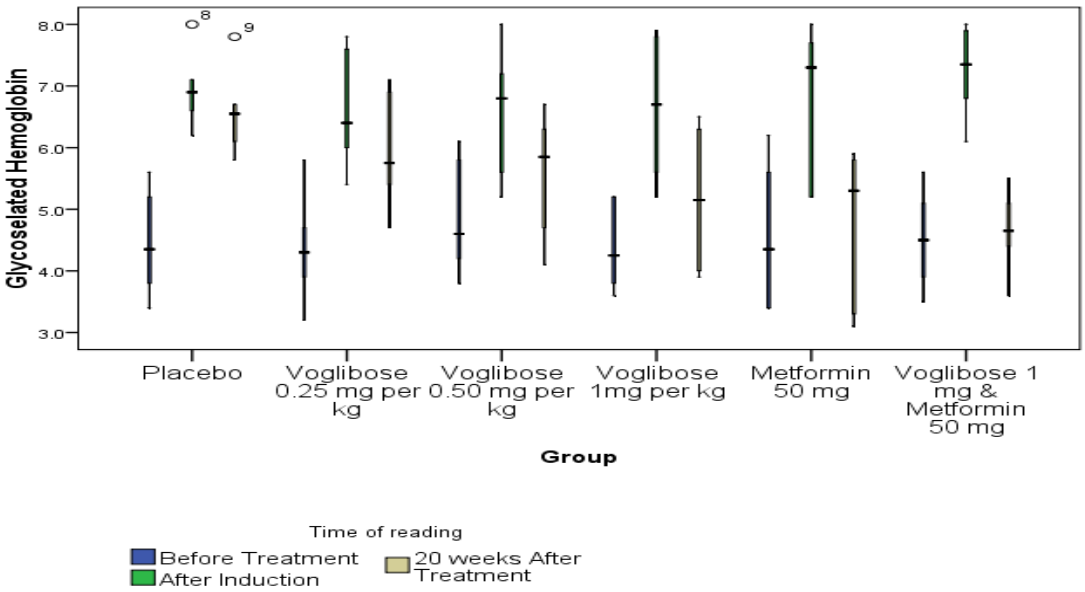

Figure 4: Comparison of distribution of Glycoselated Haemoglobin by Treatment Groups (Box Plot).

In order to precisely evaluate the effect of treatments ANOVA was conducted as the data confirms to test of normality Table3 & Table 4 presented the mean GHb before treatment, after induction and 20 wks after treatment .along with ANOVA results for different treatment groups. The mean levels of GHb with 95% of confidence interval are graphically illustrated for different groups in fig.4.Before the treatment mean GHb was in the range of 4.367 to 4.850. ANOVA reveals no significant difference in mean GHb level increased after induction and that

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was in the range of 6.60 to 7.25. There was no significant difference among treatment groups after induction (p=0.832). Twenty weeks after treatment voglibose 1 mg and metformin 50 mg exhibited the highest fall from 7.25±0.293 to 4.650±0.267 .similarly metformin 50 mg group brought about second highest level of reduction from 6.783±0.513 to 4.783±0.514. Invog 1mg/kg group there was afall from 6.650±0.452 to 5.167±0.453.In all other groups there was afall but it was less. The mean GHb level 20 weeks after treatment was significantly different among the groups with p value 0.013.

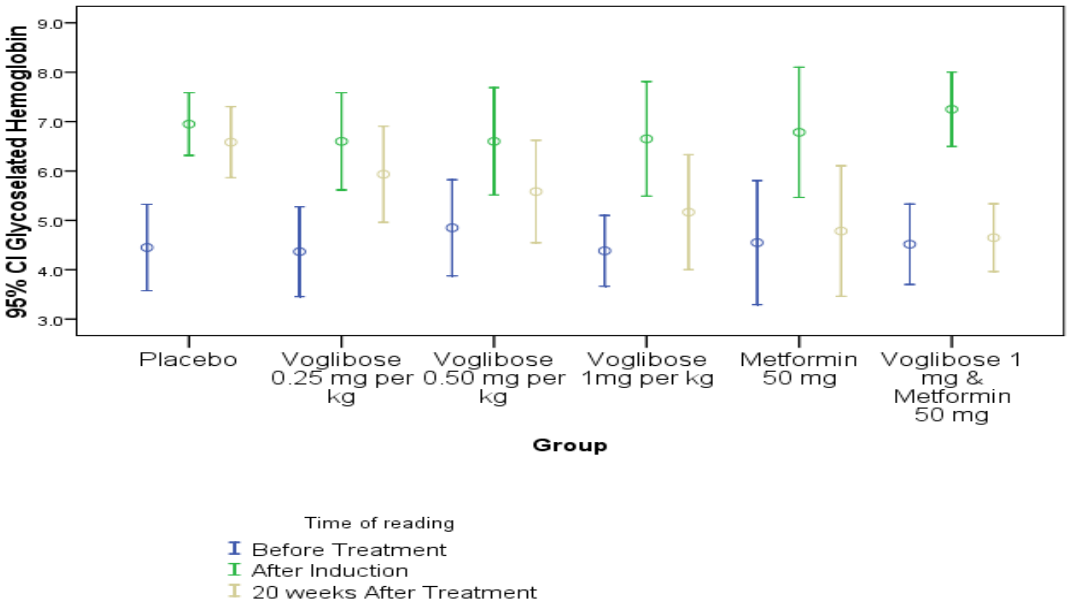

Figure 5: Comparison of Mean Glycosylated Haemoglobin along with 95% CI by Treatment Group.

Table 3: Tests of Normality for Glycosylated normality.

Shapiro-Wilk test 'p' value		
GlycosylatedHb Before Treatment	***GlycosylatedHb After Induction***	***GlycosylatedHb 20 weeks after treatment***
0.878	0.682	0.448
0.805	0.614	0.655
0.461	0.692	0.564
0.264	0.502	0.412
0.288	0.065	0.075
0.978	0.670	0.938

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Table 4: Comparison of Mean for different treatement groups.

Group	GlycosylatedHb Before Treatment	Glycosylated Hb After Induction	GlycoselatedHb 20 weeks after treatment
	Mean±SE		
Placebo	4.450±0.340	6.950±0.247	6.583±0.280
Voglibose 0.25 mg per kg	4.367±0.354	6.600±0.383	5.933±0.378
Voglibose 0.50 mg per kg	4.850±0.378	6.600±0.423	5.583±0.404
Voglibose 1mg per kg	4.383±0.279	6.650±0.452	5.167±0.453
Metformin 50 mg	4.550±0.488	6.783±0.513	4.783±0.514
Voglibose 1 mg & Metformin 50 mg	4.517±0.317	7.250±0.293	4.650±0.267
ANOVA (F Value)	0.235	0.419	3.504
(P Value)	0.944	0.832	0.013

Table 5: Pairwise Multiple Comparison of GlycosylatedHb after 20 Weeks of Treatement Between Groups.

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Placebo	Voglibose 0.25 mg per kg	0.650	0.555	1.000	-1.121	2.421
	Voglibose 0.50 mg per kg	1.000	0.555	1.000	-0.771	2.771
	Voglibose 1mg per kg	1.417	0.555	0.241	-0.354	3.187
	Metformin 50 mg	1.8000*	0.555	0.044	0.029	3.571
	Voglibose 1 mg & Metformin 50 mg	1.9333*	0.555	0.023	0.163	3.704
Voglibose 1mg per kg	Voglibose 1 mg & Metformin 50 mg	0.517	0.555	1.000	-1.254	2.287

. The mean difference is significant at the 0.05 level.

DISCUSSION

The multiple comparison of pair wise groups was done through Bonferroni Post hoc test 20 week after the treatment (Table 5). Placebo in comparison tovoglibose 0.25 mg and 0.5 mg showed no difference with very high p value.MeanGHb in vog 1 mg(5.167±0.453) in comparison to placebo (6.583±0.280) was visible lower after 20 weeks of treatment. But the pair wise comparison do not show any significant difference (p=0.241).Even though the difference is not statistically significant, the clinical effect is quite visible in case of vog 1mg in comparison to vog 0.25 and vog 0.50 mg. Effect of metformin in comparison to placebo was quite visible and was statisticaly significant (p=0.044). Similarlyvog 1mg with met 50 mg have a significant impact in comparison to placebo (p=0.023) however the combined drug met 50 mg/kg and vog 1mg/kg has the better impact than vog 1mg alone.

The effects of long term treatment with voglibose on fasting blood glucose and glycosylated Hb was studied on alloxan induced Type II DM. The study demonstrated that voglibosean α –glucosidase inhibitor can significantly lower the fasting blood glucose in alloxan induced diabetic rabbits throughout the 20 weeks. More importantly HbA1c levels also decreased with treatment of voglibose 1 mg/kg. after 20 week of treatment significantly.Again effect of combination of voglibose with metformin have a significant effect on HbA1c in comparison to placebo (p=0.023).However the combined drug voglibose and metforminhas better impact on HbA1c than voglibose, but the difference is not statistically captured.

Long standing hyperglycemia impairs insulin secretion in both IGT and Type II DM and it causes beta cell exhaustion. An improvement in glycemic control is associated with an improvement in insulin secretion [32]. One possible mechanism of effect of voglibose on FBS and HbA1c may reduce insulin secretion.This effect is beneficial in treatment of IGT and Type II DM for two reasons:1) hyperinsulinemia may be related to the development of coronary artery disease and 2) hypersecretion of insulin causes exhaustion of beta cells. Voglibose an alfaglucosidase inhibitor which inhibits alfaglucosidase and reduces PPG . The decrease in post prandial glucose may result in decrease in intrinsic insulin secretion and prevent the beta cell exhaustion. Carbohydrate absorption as well as insulin secretion is an important determinant of glycemic control. So acting by these two mechanism voglibosehave long term beneficial effect on FBS and HbA1c.

CONCLUSION

Treatment with voglibose decreases insulin secretion indirectly through delaying in intestinal absorption of carbohydrate. Voglibose can reduce the progression of Type II DM in patients with impaired glucose tolerance. So in conclusion voglibose at different doses significantly decreases FBS and HbA1c.And also voglibose can be added to metformin as an adjuvant for better glycemic control. By decreasing insulin secretion it indirectly prevent the development of coronary artery disease which is more likely to develop in patients with impaired glucose tolerance. The effect of long term treatment of voglibose is that it can prevent the progression of impaired glucose tolerance to Type II DM.

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