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ORIGINAL RESEARCH

An experimental study to evaluate the role of vitamin D and its combination with metformin in streptozotocin induced rat model of type 2 diabetes mellitus

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ABSTRACT

Background: Supplementation of vitamin D with Oral hypoglycaemics is known to reduce the levels of blood glucose. This study evaluated the role of vitamin D and its combination with metformin on blood glucose level, body weight and assess the effect on serum IL-6.

Material and Methods: An experimental study was conducted in Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow. Total of 30 wistar rats divided into 5 groups with 6 rats in each group. All rats were induced type 2 diabetes after administration of high-fat diet (HFD) for 2 weeks followed by an intraperitoneal (i.p.) injection of single low dose of streptozotocin (STZ) (35mg/Kg) dissolved in cold sodium citrate buffer (pH 4.5, 0.1 M) in a dose volume of 1ml kg−1 except the first group that served as control untreated group. A total of 6 diabetic rats treated with an oral dose of vitamin D_3 (500IU/day) with metformin (200 mg/day) for 7 weeks along with HFD comprised the Group 3 of the study while another 6 diabetic rats treated with an oral dose of vitamin D3 (100IU/day) with metformin (200mg/day) for 7 weeks along with HFD comprised Group 4 and 6 diabetic rats treated with metformin (200mg/kg) for 7 weeks along HFD who comprised Group 5 of the study.

Results: A total 6 rats were fed on normal pellet diet throughout the experiment. There was a statistically a significant difference in the blood glucose levels in this study ($p<0.05$). The intergroup comparison also shown the same. The intergroup comparison of IL-6 had also shown a significant difference ($p<0.05$). The post hoc analysis had also shown a significant difference between the groups.

Conclusion: The authors conclude that there is a significant reduction of serum glucose and IL-6 levels in rats treated with the combination of Vitamin D and Metformin than the controls.

Keywords: Metformin, Vitamin D, Streptozotocin, Type 2 Diabetes Mellitus

INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder which is characterized by elevation of plasma sugar levels.[1,2] Metformin is drug of choice for the treatment of T2DM due to its anti-hyperglycemic efficacy without causing overt hypoglycaemia. In the previous studies, it has been observed that Metformin activates the AMP-activated protein kinase (AMPK) which may confer benefits in chronic inflammatory diseases in addition to its ability to normalize blood sugar. Various previous studies have analysed its effect as an anti-inflammatory agent.[3] Vitamin D deficiency is an emerging health problem globally, which is commonly due to inadequate exposure to solar UVB radiation.[4] Recent population studies report a more prevalence of vitamin D deficiency and contributing factors being the avoidance of sun exposure, increased indoor activities and changes in dietary habits.[5] Natural dietary sources of vitamin D3 are limited so more sunlight exposure is required.[6] Vitamin D3 and vitamin D2 both have been utilized by humans.[7] According to some studies there is a clear relation between vitamin D and type 2 diabetes mellitus[8] and suggest its effect on insulin sensitivity.[9] Therefore, the present study has been designed to evaluate the role of vitamin D and its combination with metformin on blood glucose level, body weight and serum IL-6.

METHODS

The present study was conducted in the Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow after obtaining clearance from the Institutional Animal Ethics Committee (IEAC)**.**

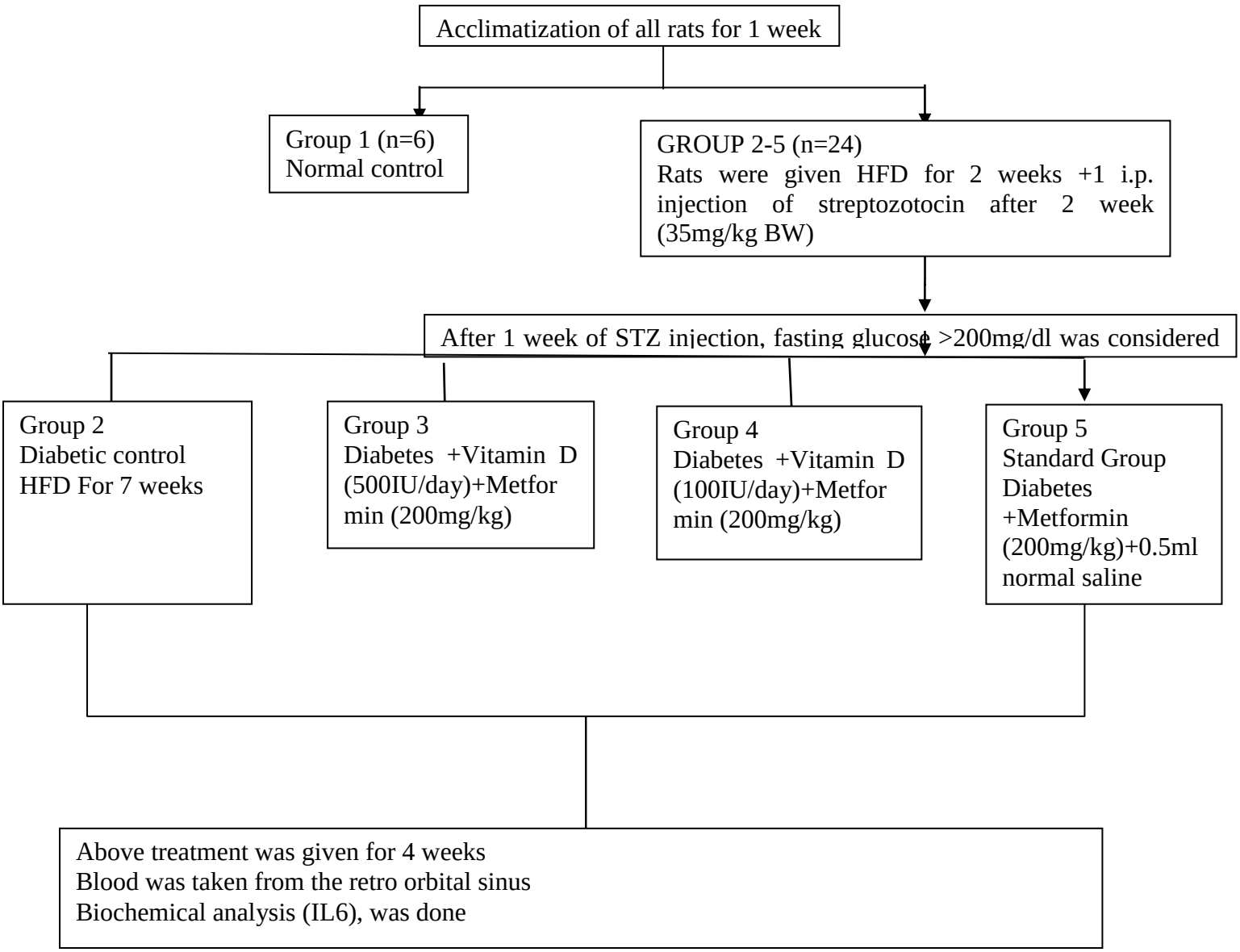
The experiments were conducted on adult wistar rats weighing 160-200gms. The animals were housed in Institutional animal house under standard conditions of housing that is room temperature of 24- 27-degree Celsius and humidity of 60-65% with 12-hour light and dark cycle. The food in the form of dry pellets was given and water was available ad libitum. In the present study, 30 experimental animals (wistar rat) were procured from CSIR-IITR, Gehru Campus, Lucknow, India.

A total number of 30 wistar rats were included in the study. They were kept in the institutional animal house under standard conditions. Rats were brought to the laboratory at least one week before the experiment and were housed separately. They were allowed to get acclimatized to the new environment for a week in cages and given free access to a high fat diet food and water ad libitum. All rats were induced type 2 diabetes after administration of high-fat diet (HFD) for 2 weeks followed by an intraperitoneal (i.p.) injection of single low dose of streptozotocin (STZ) (35mg/Kg) dissolved in cold sodium citrate buffer (pH 4.5, 0.1 M) in a dose volume of 1ml kg−1 except the first group that served as control untreated group. After one week later, all rats were tested for fasting plasma glucose and body weight. Rat with a fasting blood glucose level above 250mg/dl were considered as type 2 diabetic and included in the study. The two groups that are group 3 and group 4 were given with 500IU/rat/day of vitamin D and 100IU/rat/day for 30 days respectively.

Research methodology (flow chart showing experimental protocol)

Total animal groups = 5

Each group contained wistar rats (n=6)



The statistical analysis was done using SPSS (Statistical Package for Social Science) Version 21.0 statistical Analysis Software. The values were represented in Number (%) and

FINDINGS

For this purpose, an experimental study was carried out in which a total of 30 healthy wistar rats were randomly assigned to one of the following five groups as under (Table 1):

Table 1: Group-wise allocation of experimental animals

SN	Group		No. of experimental animals	Percentage
1	Group 1	Normal control rats fed with Normal pellet diet throughout the experiment	6	20
2	Group 2	Diabetic rats given HFD for 7 weeks orally	6	20
3	Group 3	Diabetic rats treated with Vitamin D_3 (500IU/day) and Metformin (200mg/kg/day) along with HFD FOR 7 weeks orally.	6	20

4	Group 4	Diabetic rats treated with Vitamin D_3 (100IU/day) and Metformin (200mg/kg/day) along with HFD FOR 7 weeks orally.	6	20
5	Group 5	Diabetic rats treated with Metformin(200mg/kg) orally.	6	20
	Total		**30**	

Out of 30 experimental animals, 6 (20%) were fed with Normal pellet diet throughout the experiment who comprised the Normal control group (Group 1) of the study. There were 6 STZ induced diabetic rats treated with normal saline p.o. along with HFD for 7 weeks which comprised the Group 2 of the study. A total of 6 diabetic rats treated with an oral dose of vitamin D_3 (500 IU/day) with metformin (200 IU/day) for 7 weeks along with HFD comprised the Group 3 of the study while another 6 diabetic rats treated with an oral dose of vitamin D3(100 IU/day) with metformin (200 mg/day) for 7 weeks along with HFD comprised Group 4 and 6 diabetic rats treated with metformin alone (200 mg/kg) for 7 weeks along HFD who comprised Group 5 of the study.

Table 2A: Intergroup & Intragroup Comparisons for Body Weight

Body Weight	**Group 1**		**Group 2**		**Group 3**		**Group 4**		**Group 5**		**One Way ANOVA**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F-value**	**p-value**
Baseline	158.17	28.00	177.00	22.76	169.83	20.20	155.17	15.52	173.00	14.85	1.25	0.316
2week	158.00	25.65	279.83	38.01	213.50	13.79	204.17	22.89	281.67	30.61	22.46	**<.001**
8week	158.83	25.21	287.50	29.62	175.00	20.92	173.33	24.43	129.50	15.80	38.90	**<.001**
Repeated Measures anova	F=0.393, p=0.685		F=27.06, p<**0.001**		F=18.76, p<**0.001**		F=18.08, p<**0.001**		F=90.56, p<**0.001**			

The comparison of the body weight of the rats had shown that, the mean body of group 1 rats was 158.17 gms, group 2 was 177.0 gms, group 3 was 169.83 gms, group 4 was 155.17 gms and group 5 was 173 gms. This difference in body weight was not statistically significant at the baseline (Table 2A).

After two weeks, the body weight group 2 rats was 279.83 gms which was highest among all the groups. This difference body weight was statistically significant between the groups compared.

After 8 weeks, the body weight of group 2 rats was highest and this difference was statistically significant between the groups.

The intra group of body weight comparison had shown that, there was no statistically significant difference in group1. But group 2, group 3, group 4 and group 5 had a statistically significant difference in body weight upon follow up when compared to baseline.

Table 2B: Intergroup Comparisons between Group pairs for Body Weight

Time	**Group vs Group**		**Mean Diff. (I-J)**	**SE**	**p-value**
Baseline	**Group 1**	**Group 2**	-18.83	12.03	0.532
	Group 1	**Group 3**	-11.66	12.03	0.866
	Group 1	**Group 4**	3.00	12.03	0.999
	Group 1	**Group 5**	-14.83	12.03	0.733
	Group 2	**Group 3**	7.17	12.03	0.974
	Group 2	**Group 4**	21.83	12.03	0.388
	Group 2	**Group 5**	4.00	12.03	0.997

	Group 3	**Group 4**	14.67	12.03	0.741
	Group 3	**Group 5**	-3.17	12.03	0.999
	Group 4	**Group 5**	-17.83	12.03	0.583
2week	**Group 1**	**Group 2**	-121.80	15.82	**<0.001**
	Group 1	**Group 3**	-55.50	15.82	**0.014**
	Group 1	**Group 4**	-46.16	15.82	0.052
	Group 1	**Group 5**	-123.60	15.82	**<0.001**
	Group 2	**Group 3**	66.33	15.82	**0.003**
	Group 2	**Group 4**	75.67	15.82	**0.001**
	Group 2	**Group 5**	-1.83	15.82	1.000
	Group 3	**Group 4**	9.33	15.82	0.975
	Group 3	**Group 5**	-68.16	15.82	**0.002**
	Group 4	**Group 5**	-77.50	15.82	**<0.001**
8week	**Group 1**	**Group 2**	-128.60	13.66	**<0.001**
	Group 1	**Group 3**	-16.16	13.66	0.760
	Group 1	**Group 4**	-14.50	13.66	0.824
	Group 1	**Group 5**	29.33	13.66	0.232
	Group 2	**Group 3**	112.50	13.66	**<0.001**
	Group 2	**Group 4**	114.16	13.66	**<0.001**
	Group 2	**Group 5**	158.00	13.66	**<0.001**
	Group 3	**Group 4**	1.67	13.66	1.000
	Group 3	**Group 5**	45.50	13.66	**0.021**
	Group 4	**Group 5**	43.83	13.66	**0.027**

The intergroup comparisons of body weight had shown that, there was no statistically significant difference in body weights at the baseline between any groups. At 2 weeks of follow up, there was a statistically significant difference in body weight between group 1 & 2, 1& 3, 1& 5, 2 & 3, 2 & 4 and 3 & 5. At 8 weeks of follow up, statistically significant difference was seen between 1 & 2, 2 & 3, 2 & 3, 2 & 4, 2 & 5, 3 & 5 and 4 & 5 (Table 2B).

Table 3A: Intergroup & Intragroup Comparisons for Glucose

Glucose	**Group 1**		**Group 2**		**Group 3**		**Group 4**		**Group 5**		**One Way ANOVA**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F-value**	**p-value**
Baseline	72.67	8.64	78.83	14.25	71.50	6.66	68.83	6.91	55.83	3.76	5.63	**0.002**
2week	69.17	12.04	296.67	28.88	286.17	54.36	231.83	39.54	305.00	40.66	40.69	**<.001**
8week	68.00	10.55	296.67	29.01	137.67	38.51	134.33	28.70	89.50	7.15	72.92	**<.001**
Repeated Measures anova	F=1.78, p=0.219		F=217.44, p<0.001		F=38.87, p<**0.001**		F=40.40, p<**0.001**		F=181.52, p<**0.001**			

Table 3B: Intergroup Comparisons between Group pairs for Glucose

Time	**Group vs Group**		**Mean Diff. (I-J)**	**SE**	**p-value**
Baseline	**Group 1**	**Group 2**	-6.17	5.06	0.741
	Group 1	**Group 3**	1.17	5.06	0.999
	Group 1	**Group 4**	3.83	5.06	0.940
	Group 1	**Group 5**	16.83	5.06	**0.021**
	Group 2	**Group 3**	7.33	5.06	0.603

	Group 2	**Group 4**	10.00	5.06	0.306
	Group 2	**Group 5**	23.00	5.06	**0.001**
	Group 3	**Group 4**	2.67	5.06	0.984
	Group 3	**Group 5**	15.67	5.06	**0.035**
	Group 4	**Group 5**	13.00	5.06	0.107
2week	**Group 1**	**Group 2**	-227.50	21.83	**<0.001**
	Group 1	**Group 3**	-217.00	21.83	**<0.001**
	Group 1	**Group 4**	-162.60	21.83	**<0.001**
	Group 1	**Group 5**	-235.80	21.83	**<0.001**
	Group 2	**Group 3**	10.50	21.83	0.988
	Group 2	**Group 4**	64.83	21.83	**0.047**
	Group 2	**Group 5**	-8.33	21.83	0.995
	Group 3	**Group 4**	54.33	21.83	0.125
	Group 3	**Group 5**	-18.83	21.83	0.908
	Group 4	**Group 5**	-73.16	21.83	**0.020**
8week	**Group 1**	**Group 2**	-228.60	14.85	**<0.001**
	Group 1	**Group 3**	-69.66	14.85	**0.001**
	Group 1	**Group 4**	-66.33	14.85	**0.001**
	Group 1	**Group 5**	-21.50	14.85	0.604
	Group 2	**Group 3**	159.00	14.85	**<0.001**
	Group 2	**Group 4**	162.33	14.85	**<0.001**
	Group 2	**Group 5**	207.16	14.85	**<0.001**
	Group 3	**Group 4**	3.33	14.85	0.999
	Group 3	**Group 5**	48.17	14.85	**0.025**
	Group 4	**Group 5**	44.83	14.85	**0.042**

At baseline mean blood sugar level of Group 5 (55.83 mg/dl) was found to be minimum while maximum blood sugar level was observed for Group 2 (78.83 mg/dl). On comparing intergroup difference, none of the difference was found to be significant.

At 2 weeks, blood sugar of Group 1 animals (69.17 mg/dl) was found to be significantly lower than that of rest of the groups. Blood sugar levels of Group 2,3,4,5 were comparable.

At 8 weeks, minimum Blood sugar level was observed in Group 1(68.0 mg/dl) followed by Group 5 (89.50 mg/dl) and Group 4 (134.33 mg/dl) while maximum blood sugar levels was observed in Group 2 (296.67 mg/dl) followed by Group 3 (137.67mg/dl). Significant difference in blood sugar levels of above five groups wereobserved. On exploring intergroup comparison all of the differences were found to be significant. Maximum difference was observed between 1 and 2 (228.60+/- mg/dl) followed by between Group 2 and Group 5 (207.16 mg/dl) and Group 2 and Group 4 (162.33mg/dl),Group 2 and Group 3(159 mg/dl) while minimum difference was observed between Group 3 and Group 4 (3.33mg/dl) followed by Group 1 and Group 5 (21.5 mg/dl).Order of Blood sugar levels in study population was Group 2 > Group 3 > Group 4 >Group 5 > Group 1.

Rise in baseline blood sugar level was observed in all groups at 3rd week. Changes were significant in all the groups except Group 1.Decline in blood sugar levels were observed during 3rd week to 8th week in all the groups except Group 2 where increment in blood sugar level was observed. Changes in blood sugar levels of 3rd to 8th were not significant in Group 1 and Group 2. All the groups had blood sugar levels above baseline at 8th weeks, though the difference was not significant in Group 1 (Table 3A and 3B).

Table 4A : Intergroup Comparisons for IL-6

IL-6	Group 1		Group 2		Group 3		Group 4		Group 5		One Way ANOVA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F-value	p-value
8week	24.89	4.4	31.9	10.3	15.09	2.06	25.04	4.68	27.05	5.21	6.28	**<.001**

The mean IL-6 was higher for group 5 of rats and lower for group 3 rats in this study. The mean difference in IL-6 was statistically significant between the groups.

Table 4B: Intergroup Comparisons between Group pairs for IL-6

Time	Group vs Group		Mean Diff. (I-J)	SE	p-value
8 weeks	**Group 1**	**Group 2**	-7.015	3.45	0.053
	Group 1	**Group 3**	-9.798	3.45	**0.009**
	Group 1	**Group 4**	-0.15	3.45	0.966
	Group 1	**Group 5**	-2.165	3.45	0.537
	Group 2	**Group 3**	16.8133	3.45	**0.000**
	Group 2	**Group 4**	6.865	3.45	0.058
	Group 2	**Group 5**	4.85	3.45	0.173
	Group 3	**Group 4**	-9.9483	3.45	**0.008**
	Group 3	**Group 5**	-11.9633	3.45	**0.002**
	Group 4	**Group 5**	-2.015	3.45	0.565

The intergroup comparison of IL – 6 was statistically significant between group 1 & group 3, group 2 & 3, group 3 & 4 and group 3 & 5 (Table 4A and 4B) .

DISCUSSION

Diabetes mellitus (DM) is a chronic metabolic disorder often characterized by higher and persistent elevated blood sugar levels.[1, 2]

This study was conducted in the Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow in order to evaluate the effect of Vitamin D combined with Metformin in streptozotocin induced diabetes mellitus in wistar rats.

About 20% of the experimental animals were fed with Normal pellet diet throughout the experiment who comprised the Normal control group (Group 1), 20% of the rats had STZ induced diabetes treated with normal saline p.o. along with HFD for 7 weeks which comprised the Group 2, 20% of diabetic rats treated with an oral dose of vitamin D_3 (500 IU/day) with metformin (200 IU/day) for 7 weeks along with HFD comprised the Group 3, while another 20% of the diabetic rats treated with an oral dose of vitamin D3 (100 IU/day) with metformin (200 mg/day) for 7 weeks along with HFD comprised Group 4 and 6 diabetic rats treated with metformin (200 mg/kg) for 7 weeks along HFD who comprised Group 5 of the study.

BLOOD SUGAR LEVELS OF GROUPS

At baseline mean blood sugar level of Group 5 (55.83 mg/dl) was found to be minimum while maximum blood sugar level was observed for Group 2 (78.83 mg/dl). On comparing intergroup difference, none of the difference was found to be significant. At 2 weeks of follow up, blood sugar of Group 1 animals (69.17 mg/dl) was found to be significantly lower than that of rest of the groups. Blood sugar levels of Group 2,3,4,5 were comparable. At 8 weeks of follow up, minimum Blood sugar level was observed in Group 1 (68.0 mg/dl) followed by Group 5 (89.50 mg/dl) and Group 4 (134.33 mg/dl) while maximum blood sugar

levels was observed in Group 2 (296.67 mg/dl) followed by Group 3 (137.67 mg/dl). Significant difference in blood sugar level of above five groups were observed. On exploring intergroup comparison all of the differences were found to be significant. Maximum difference was observed between 1 and 2 (228.60+/- mg/dl) followed by between Group 2 and Group 5 (207.16 mg/dl) and Group 2 and Group 4 (162.33 mg/dl), Group 2 and Group 3(159 mg/dl) while minimum difference was observed between Group 3 and Group 4 (3.33 mg/dl) followed by Group 1 and Group 5 (21.5 mg/dl). Order of Blood sugar levels in study population was Group 2 > Group 3 > Group 4 >Group 5 > Group 1.

Rise in baseline blood sugar level was observed in all groups at 3rd week. Changes were significant in all the groups except Group 1. Decline in blood sugar levels were observed during 3rd week to 8th week in all the groups except Group 2 where increment in blood sugar level was observed. Changes in blood sugar levels of 3rd to 8thweek were not significant in Group 1 and Group 2. All the groups had blood sugar levels above baseline at 8th weeks of follow up, though the difference was not significant in Group 1.

In a study by Kabel et al, after 12 weeks of follow up, the serum fasting glucose in control group was 78.26 mg/dl, in STZ group was 215.63 mg/dl, STZ with metformin group was 147.4 mg/dl, STZ with vitamin D group was 175.35 mg/dl, STZ with peanut oil group was 207.2 mg/dl and STZ with metformin with vitamin D was 110.82 mg/dl. The administration of vitamin D or metformin alone induced significant decrease in serum fasting glucose.[10] In our study vitamin D and metformin combination induced significant decrease in serum fasting glucose.

In a study by Abdel Rahim et al, alfacalcidiol, solely or combined with metformin, significantly help in correction of glucose homeostasis and lipid profile parameters. Alfacalcidiol alone or in combination with metformin helps in downregulation of mRNA expression of SREBP-1c in the liver. Vitamin D3 helps to modulate glucose and lipid metabolism in diabetic rats and also provide additional protective effects when combined with metformin.[11]

Oral vitamin D alone or in combination with metformin improve insulin sensitivity in skeletal muscles and beneficial in insulin resistance.[12]

INTER GROUP COMPARISON OF IL – 6

The intergroup comparison of IL-6 had shown a statistically significant difference between the groups of rats used. IL – 6 was lower for the group of rats treated with Vitamin D3 (500IU/days) and Metformin (200mg/kg/day) along with HFD FOR 7 weeks orally. The inflammation have a crucial role in diabetes induced macrovascular and micro vascular complications.[11]

CONCLUSION

This study has shown a significant reduction of serum glucose levels in rats treated with the combination of Vitamin D and Metformin as compared to metformin group alone. IL-6 is a marker for inflammation and in our study we found that Vitamin D and metformin combination treated rats were having lesser value of IL-6 as compared to metformin alone.

CONFLICTS OF INTEREST

There is no potential conflicts of interest for any of the authours.

FINANCIAL ASSISTANCE

There is no financial assistance for this study.

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