Original Article

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Pharmacology Section

Effect of Rosuvastatin and Glibenclamide in Alone or in Combination on Glucose Homeostasis in Diabetic Male Albino Wistar Rats: An Observer-blinded Experimental Interventional Study

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ABSTRACT

Introduction: Metabolic Syndrome (MetS) is a complex of metabolic disorders which include obesity, insulin resistance, hyperglycaemia, hypertension along with dyslipidaemia. It is not clear whether rosuvastatin is having a role in new onset diabetes.

Aim: To assess the effect of rosuvastatin in comparison to glibenclamide on blood sugar levels and insulin resistance in diabetic rats.

Materials and Methods: This experimental study was carried out at Sri Manakula Vinayagar Medical College and Hospital, Kalitheerthalkuppam, Puducherry on 42 adult male Wistar rats, for eight weeks. Diabetes was created by feeding rats with High Fat and High Sugar (HFHS) diet over a period of eight weeks. In total, 42 rats were allocated to seven groups, consisting of six animals per group. Group I- vehicle treated animals (control), group II- HFHS diet animals (Diabetic Control- DC), group III- Glibenclamide (G) (5 mg/kg) + HFHS, group IV-rosuvastatin (R) (5 mg/kg) + HFHS, group V-R (10 mg/kg) + HFHS, group VI-G (5 mg/kg) + R (5 mg/kg) + HFHS diet, group VII-G (5 mg/kg) + R (10 mg/kg) + HFHS diet continued for four weeks. Rosuvastatin and glibenclamide were dissolved in distilled water and 95% ethanol respectively, and were given orally daily for four weeks after induction of diabetes. Data were subjected to one-way ANOVA using the Statistical Package for the Social Sciences (SPSS) version 24.0 followed by non parametric test with significance level set at $p<0.05$.

Results: By end of 8th week, on comparison of control group with HFHS diet and glibenclamide treated group, there was significant rise in body weight, serum Low Density Lipoprotein (LDL), in addition to Very Low Density Lipoprotein (VLDL) and Triglyceride (TG) levels. Glibenclamide treated (group III) animals on comparison with groups IV, V, VI, VII revealed reduction in body weight, serum LDL, VLDL and TG levels ($p<0.001$). Glibenclamide treated (group III) on comparing with groups IV, V, VI, VII, there was significant high serum High Density Lipoprotein (HDL) level ($p<0.001$). By 8th week, significant decrease in serum insulin and Homeostasis Model Assessment-estimated Insulin Resistance (HOMA-IR) level was observed when glibenclamide group III was compared with groups IV to VII treated animals ($p<0.001$).

Conclusion: The study results indicate that the combination therapy of rosuvastatin and glibenclamide demonstrate beneficial effects on weight reduction, lipid profile and insulin resistance.

Keywords: Cholesterol, Diabetes, Insulin resistance, Lipid profile

INTRODUCTION

Metabolic syndrome (MetS) consists of metabolic disorders which include obesity, insulin resistance, hyperglycaemia, hypertension along with dyslipidaemia which is on the rise worldwide [1]. Its prevalence is high especially among Asians, including Indians. In India, it ranges from 16.3% to as high as 48.2%. It is a critical risk factor accountable for Type 2 Diabetes Mellitus (T2DM) and Cardiovascular Diseases (CVD), which are responsible for increased mortality and morbidity [2].

Most subjects with MetS receive statins to manage dyslipidaemia. Statins act by inhibition of 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase. They are used to treat the dyslipidaemia component of MetS. Additionally, they demonstrate several pleiotropic actions like improvement in endothelial function in atherosclerosis [3,4]. Several clinical studies ascertained that statins are beneficial in reduction of atherosclerosis and have favourable influence in diabetic patients [4]. On the contrary, statins are also considered to have a role in newly onset T2DM, although uncertain whether by reducing the insulin secretion [5].

A recent meta-analysis revealed amplified risk of development of diabetes with the intake of statins [5]. The proposed mechanism for the augmented risk of diabetes with statins because of enhanced formation of plasma-derived Low Density Lipoprotein (LDL) cholesterol as a result of statin-induced antagonism of cholesterol production which results in direct inflammatory changes and oxidation in beta cells, in turn resulting in cellular apoptosis and impaired insulin secretion. Statins also exert influence on glucose metabolism and Insulin Resistance (IR); the probable mechanism could be reduction in insulin secretion [6,7]. The other likely causes of pathogenesis includes the effect of statins in HMG-CoA reductase inhibition, calcium release, isoprenoid synthesis, glucose transport, calcium mediated pancreatic insulin secretion, lowering of different isoprenoids [8]. Hence, it is uncertain whether statins can certainly control Blood Sugar Levels (BSL) in diabetic patients, or even in the diabetic model animals.

Since statins are commonly used in prevention of CVD in diabetes and non diabetic patients, it is important to evaluate the effect

of statin intake BSL and insulin resistance. Current therapeutic strategies for T2DM are limited and it involves insulin and oral hypoglycaemic drugs. Sulfonylureas are rapidly acting class of drugs which stimulate pancreatic insulin secretion e.g., Rosuvastatin, Glibenclamide and Glipizide. Rosuvastatin is a commonly used statin to control dyslipidaemia and reduce the risk of CVD. Therefore, this experimental study was carried out to evaluate effectiveness of rosuvastatin in controlling the diabetes and IR in diabetic rats as compared to glibenclamide.

MATERIALS AND METHODS

This observer-blinded experimental study was carried out at Sri Manakula Vinayagar Medical College and Hospital, Kalitheerthalkuppam, Puducherry, between 2016 to 2019. The study was carried out after the protocol was approved by Institutional Animal Ethics Committee (Approval letter No. VMMCH/2016/IAEC/21). Adult male Wistar rats of age 10-12 weeks and weight 120-180 g were obtained from the King's Institute Guindy, Chennai. Unhealthy animals were excluded from the study.

Procedure

The experiment was carried out in the light hours between 10.00 and 12.00 hours. The animals were cared and maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and Good Laboratory Practices (GLP).

Animals were given HFHS diet daily for a period for eight weeks to induce diabetes. Rats were administered high fat by mixing [commercially available Vanaspati ghee (vegetable oil) and coconut oil] in the ratio of 3:1 (v/v). The dose given was 3 mL/kg body weight/day oral diet and high sugar diet consisting of 25% fructose (which means that for each 100 mL water, 25 g of fructose was added) in drinking water and fed over 24 hours for eight weeks. Rats were weighed prior, during and at the end of the experimental procedure. Total duration of study was six weeks [9].

The following chemicals and drugs were used as high fat (mixing vegetable oil + coconut oil of 3:1 ratio)- 3 mL/kg and high sugar; Diet (25% fructose for 24 hours along with water), Glibenclamide (G) (5 mg/kg/day), Rosuvastatin (R) (5 and 10 mg/kg/day), 95% ethanol, distilled water and appropriate vehicle was used for control animals.

Six mice per group are sufficient for both the statistical significance and adherence to the rule of investing mice in experimentation. In total, 42 animals are required for test and they were divided into seven groups consisting 6 animals as follows:

Group I: Vehicle treated animals (control)

Group II: HFHS diet animals - DC

Group III: G (5 mg/kg) + HFHS

Group IV: R (5 mg/kg) + HFHS

Group V: R (10 mg/kg) + HFHS

Group VI: G (5 mg/kg) + R (5 mg/kg) + HFHS diet

Group VII: G (5 mg/kg) + R (10 mg/kg) + HFHS diet

Rosuvastatin and glibenclamide were dissolved in distilled water and 95% ethanol respectively, and were given orally daily for four weeks after induction of diabetes and were continued for four weeks.

Experimental procedure: The drugs and diet were given for a period of eight weeks. On 4th and 8th week of experiment after overnight fasting, rats were given anaesthesia with small quantity of ether. Blood was obtained through retro-orbital puncture technique. Blood glucose, lipid profile and insulin levels were measured using the blood sample [10].

Blood glucose was measured with Accu-Chek (R) glucometer (by Roche Diabetes Care, Inc.,) after the collection of blood sample from the all night (12-15 hour) fasted rats on every week of experiment. Body weight gain or loss in each experimental rat were measured and recorded on every week with triple balance [9]. Following lipid parameters were measured and recorded on 4th and 8th week

i. Serum Total Cholesterol (TC)

ii. Serum Triglycerides (TG)

iii. Serum High Density Lipoproteins (HDL)

iv. Serum Low Density Lipoproteins (LDL)

v. Serum Very Low Density Lipoproteins (VLDL)

Fasting serum insulin measurement and Homeostasis Model Assessment-estimated Insulin Resistance (HOMA-IR) were calculated.

STATISTICAL ANALYSIS

Data were subjected to one-way ANOVA which was followed with Post-Hoc Tukey's test using the Statistical Package for the Social Sciences (SPSS) software version 24.0 followed by non parametric test with significance level set at $p<0.05$.

RESULTS

The effect of rosuvastatin and glibenclamide (alone and in combination) on body weight in euglycaemic and diabetes induced male albino Wistar rats are summarised in [Table/Fig-1].

On 0th week and at end of 1st week, significant differences was not observed in weight of rats on comparison of control with

Treatment (mg/kg)	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control (Distilled water)	160.5±5.9	160.3±4.6	158.1±4.2	159.2±3.1	161.0±3.6	161.9±3.6	162.7±5.2	164.0±6.1	165.9±6.9
Diabetic control (HFHS diet)	169.9±4.7	169.0±3.6	182.4±12.2***	188.4±14.8**	218±42.6***	227.5±52.9***	249.9±60.0***	272.7±70.9***	295.7±73.4***
G (5 mg/kg) + HFHS	170.0±3.8	169.0±6.2	168.0±10.5***	170.5±20.7***	271.7±4.1***###	170.0±21.5*	164.0±21.9	167.0±20.5##	261.4±8.6***
R (5 mg/kg) + HFHS	167.1±68.2	168.0±67.7	170.5±69.5$	183.0±73.9*$$	199.0±80.0*#$$$	180.6±76.9	160.0±72.5###	160.0±68.4###	167.0±67.2###$$$
R (10 mg/kg) + HFHS	166.8±1.5	169.9±5.2	177.3±6.5**	185.1±9.7**$$	202.9±16.8**$$$	193.5±14.9	155.8±23.8###$	155.8±17.3###	159.0±1.8###$$$
G (5 mg/kg) + R (5 mg/kg) + HFHS diet	167.3±2.2	168.1±6.8	180.7±10.3***	191.5±12.7***	203.7±17.3**$$$	189.9±17.1	159.9±15.9###	159.9±8.7###	162.9±5.5###$$$
G (5 mg/kg) + R (10 mg/kg) + HFHS diet	168.9±6.9	166.7±9.1	180.5±6.5***	194.2±9.9***	206.9±9.9***$$$	186.2±23.5	152.8±22.8###$	152.4±12.8###$	149.9±7.1###$$$

[Table/Fig-1]: Effect of Rosuvastatin and Glibenclamide (alone and in combination) on body weight in euglycaemic and diabetes induced male albino Wistar rats.
Rosuvastatin (R), Glibenclamide (G)
[Values expressed as Mean±SD (n = 6 in each group)]
*p<0.05, **p<0.01,***p<0.001 in comparison to vehicle treated control group
#p<0.05,##p<0.01, ###p<0.001 in comparison to HFHS diet diabetic control group
$P<0.05, $$p<0.01, $$$p<0.001 in comparison to HFHS diet Glibenclamide treated group
Evaluation was carried out by one-way ANOVA which was followed with Post-Hoc Tukey's test

different groups. By end of 8th week, when control (group I) was compared with HFHS diet (group II) and glibenclamide treated (group III) there was significant rise in body weight (p<0.001). Glibenclamide (5 mg/kg) treated animals (group III) compared with groups IV, V, VI, VII showed significant decrease in body weight (p<0.001).

The effect of rosuvastatin and glibenclamide (alone and in combination) on serum Fasting Blood Sugar (FBS) level in euglycaemic and diabetes induced male albino Wistar rats have been summarised in [Table/Fig-2].

At 0th week and at the end of 1st week, significant changes were not observed in serum fasting BSLs. At end of 8th week when on comparison of normal control (group I) with group- II, III, IV, V, VI, VII significant rise in FBS level (p<0.001) was observed. There was statistical decrease in blood glucose level on comparing HFHS diet animals (group II) with groups III, IV, V (p<0.001, p<0.01, p<0.001). However, on comparison of glibenclamide (5 mg/kg) group of animals with groups IV, V, VI, and VII there was statistical reduction in serum FBS level (p<0.05, p<0.05, p<0.01, p<0.001) respectively.

The effect of rosuvastatin and glibenclamide (alone and in combination) on lipid profile in euglycaemic and diabetes induced male albino Wistar rats is summarised in [Table/Fig-3].

Serum Total Cholesterol level (TC): By end of 4th week, significant rise in serum TC level was observed on comparison of normal control (group I) with groups II to VII (p<0.001).

By end of 8th week, on comparison of normal control (group I) with groups II, III, IV, V, VI, VII treated rats there was significant rise in levels of serum TC (p<0.001). However, significant reduction in serum TC level was observed in groups III, IV, V, VI, VII of animals (p<0.001) when compared with group II animals. On comparison of glibenclamide (5 mg/kg) treated (group III) with other groups IV, V, VI, VII treated animals there was significant reduction in TC level (p<0.001).

Serum Triglyceride level (TG): By end of 4th week, significant rise in serum TG level was observed in normal control (group I) compared to groups II, III, IV, V, VI, VII treated animals (p<0.001). Comparison of HFHS diet (group II) with group III, IV, V, VII showed significant increased levels of serum TG (p<0.01, p<0.01, p<0.05, p<0.01) respectively. However when glibenclamide (5 mg/kg) (group III) was compared with groups V, VI, VII, it showed significant increase in serum TG level (p<0.05, p<0.01, p<0.001) respectively.

On 8th week, when normal group was compared with groups II, III, IV, V, VI, VII treated animals showed statistically significant increase in serum TG level (p<0.001). However, HFHS diet (group II) when compared with groups III, IV, V, VI, VII there was significant decreased levels of serum TG level (p<0.001). Glibenclamide (5 mg/kg) treated (group III) on comparing groups IV, V, VI, VII treated animals there was significant reduction in serum TG level.

Serum HDL level: On 4th week, observed a significant decrease in serum HDL level when compared normal (group I), with groups II to VII treated animals. Glibenclamide (5 mg/kg) on comparing with group V treated animals there was slight increase in the HDL level (p<0.05).

Treatment (mg/kg)	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control (Distilled water)	95.1±3.5	95.2±3.2	93.4±3.4	92.8±3.6	93.3±3.7	92.9±3.5	92.6±2.7	92.2±4.7	94.1±4.9
Diabetic control (HFHS diet)	96.1±5.2	95.8±4.8	105.1±4.1*	119.2±7.2***	130.0±4.2***	133.6±9.2***	138.4±4.9***	145.7±10.3***	148.6±10.3***
G (5 mg/kg) + HFHS	97.4	98.0±4.6	111.5±5.1***	122.3±3.0***	128.7±2.0***	126.8±2.5***###	125.9±2.3***###	124.7±1.9***###	123.8±3.1***###
R (5 mg/kg) + HFHS	93.4±4.0	99.6±6.9	114.5±7.9***	129.5±5.2***##	137.3±3.4***##$$$	136.0±3.3***$	136.4±1.7***$	135.6±2.2***#$	135.5±3.3***##$
R (10 mg/kg) + HFHS	94.9±5	103.2±4.9	114.3±8.1***	127.2±5.5***	133.8±2.9***	132.2±4.9***	131.4±3.9***	130.2±5.1***###	130.9±4.2***###$
G (5 mg/kg) + R (5 mg/kg) + HFHS diet	91.8±8.8	93.1±8.3	110.9±5.7***	124.5±3.6***	133.4±3.5***	130.6±3.1***	124.3±3.9***###	120.9±6.0***###	110.3±6.9***$$
G (5 mg/kg) + R (10 mg/kg) + HFHS diet	94.9±4.8	96.6±4.6	108.6±5.9**	124.3±3.4***	130.1±2.4***	127.7±2.5***	123.9±2.5***###	111.8±3.2***###$$	105.2±1.8***$$$

[Table/Fig-2]: Effect of Rosuvastatin and Glibenclamide (alone and in combination) on serum Fasting Blood Glucose (FBS) level in euglycaemic and diabetes induced male albino Wistar rats.
Rosuvastatin (R), Glibenclamide (G)
[Values expressed as Mean±SD (n = 6 in each group)]
*p<0.05,**p<0.01,***p<0.001 in comparison to vehicle treated control group
#p<0.05,##p<0.01,###p<0.001 as comparison to with HFHS diet diabetic control group
$p<0.05,$$p<0.01,$$$p<0.001 as compared with HFHS diet Glibenclamide treated group
Evaluation was carried out by one-way ANOVA which was followed with Post-Hoc Tukey's test

	TC		TG		HDL		VLDL		LDL	
Treatment (mg/kg)	Week 4	Week 8	Week 4	Week 8	Week 4	Week 8	Week 4	Week 8	Week 4	Week 8
Control (Distilled water)	64.4±2.2	65.0±2.2	72.1±1.7	73.5±1.3	32.0±0.8	32.5±1.4	14.4±0.3	15.5±0.3	18.0±0.5	18.5±0.5
Diabetic control (HFHS diet)	73.1±1.5***	97.6±1.2***	225.1±3.4***	300.1± 20.8***	28.5± 0.7***	25.0±0.8***	45.0±0.7***	60±4.2***	27.0±1.3***	27.1±1.0***
G (5 mg/kg) + HFHS	73.9±1.0***	92.9± 0.8****###	220.6± 0.7****#	200.7± 2.3****###	27.5±1.1***	28.5±1.3****#	43.6±1.8****#	40.1± 0.5****###	28.5±0.6****#	27.1± 0.7****###
R (5 mg/kg) + HFHS	74.1±2.2***	68.2± 1.5****###$$$	218.7± 0.7****###	166.4± 1.9****###$$$	27.6±0.6***	32.0± 1.1****###$$	43.7±0.1***	33.3± 0.4****###$$$	28.9±0.5****#	25.5± 0.9****###$$$
R (10 mg/kg) + HFHS	73.6±1.1***	50.5± 0.8****###$$$	230.1± 1.0****$$$	148.0± 0.8****###$$$	29.2±0.7***$	37.0± 2.8****###$$$	46.0±0.2****###	29.6± 0.2****###$$$	28.5±0.6***$$$	24.6± 01.4****###$$$
G (5 mg/kg) + R (5 mg/kg) + HFHS diet	74.3±1.1***	67.4± 0.5****###$$$	225.1± 0.9****$$	145.8± 0.5****###$$$	28.1±1.0***	35.0± 0.9###$$$	45.0±0.2***$	29.2± 0.1****###$$$	27.3±0.9***	25.0± 0.7****###$$$
G (5 mg/kg) + R (10 mg/kg) + HFHS diet	73.2±2.1***	46.3± 0.7****###$$$	232.3± 1.9****###$$$	151.5± 2.6****###$$$	28.1±0.7***	40.5± 0.7****###$$$	46.5± 0.4****$$$	30.3± 0.5****###$$$	28.2± 0.7****###$$$	24.1± 0.4****###$$$

[Table/Fig-3]: Effect of Rosuvastatin and Glibenclamide (alone and in combination) on lipid profile in euglycaemic and diabetes induced male albino Wistar rats.
Rosuvastatin (R), Glibenclamide (G)
[Values expressed as Mean±SD (n = 6 in each group)]
*p<0.05,**p<0.01,***p<0.001 in comparison to vehicle treated control group
#p<0.05,##p<0.01,###p<0.001 as comparison to with HFHS diet diabetic control group
$p<0.05,$$p<0.01,$$$p<0.001 as compared with HFHS diet Glibenclamide treated group
Evaluation was carried out by one-way ANOVA which was followed with Post-Hoc Tukey's test

On 8th week, when normal group compared with group II, III, IV treated animals showed statistically significant decrease (p<0.001) and groups V, VII showed an increase in serum HDL level (p<0.001). However, HFHS diet when compared with groups III to VII there was significant increased levels of serum HDL level (p<0.001). Glibenclamide (5 mg/kg) treated (group III) on comparing groups IV to VII treated animals there was significant high serum HDL level (p<0.001).

Serum VLDL: At the end of 4th week there was significant rise in VLDL level when compared with control (group I) with groups II, III, IV, V, VI, and VII treated animals (p<0.001). On comparing HFHS diet group animals with groups III, V, and VII observed significant increase in serum VLDL level (p<0.05, p<0.001, p<0.05). Also observed significant increased levels of VLDL when compared to glibenclamide (5 mg/kg) with groups VI, VII (p<0.001).

At the end of 8th week, VLDL level was significantly increased in groups II, III, IV, V, VI, and VII treated animals when compared with normal group (p<0.001). But observed significant reduced levels of VLDL on comparison of HFHS diet (group II) with groups III, IV, V, VI, VII treated animals (p<0.001). Also, there was significant decrease in VLDL level in groups IV, V, VI, and VII when compared with glibenclamide (5mg/kg) treated group (p<0.001).

Serum LDL level: At the end of 4th week there was significant increase in LDL level when compared to control with groups II, III, IV, V, VI, VII treated animals (p<0.001). HFHS diet (group III) animals on comparing with groups III, IV, VII there was significant raised levels of serum LDL (p<0.01, p<0.01, p<0.001). When the glibenclamide group (5 mg/kg) was compared with groups V, and VII treated animals there was significant increase in the LDL level (p<0.001).

At the end of 8th week, when serum LDL level was compared between control with groups II, III, IV, V, VI, VII there was significant rise in serum LDL value (p<0.001). But observed a significant reduction in LDL level on comparing group II with groups III, IV, V, VI, and VII (p<0.001). Also there was significant decrease in LDL level with groups IV, V, VI, and VII when compared with glibenclamide (5mg/kg) treated group (p<0.001).

On 4th week there was significant rise in serum insulin level and HOMA-IR on comparison of normal control with groups II to VII treated animals (p<0.001). HFHS diet group animals when compared with groups VII observed significant increase in serum insulin level (p<0.01). However, HOMA-IR levels were increased with groups IV, V, VII (p<0.001, p<0.05, p<0.01). Glibenclamide (5 mg/kg) group when compared with group VI and group IV there was significant increase in the insulin [(p<0.01) and (p<0.05), respectively] and HOMA-IR [Table/Fig-4].

By end of 8th week, there was significant increased levels of insulin and HOMA-IR level when control group was compared with groups II to VII treated animals (p<0.001). When HFHS diet animals were compared with groups III to VII treated animals there was significant reduction in insulin level and HOMA-IR (group I). Significant decrease in serum insulin and HOMA-IR level was observed when glibenclamide (5 mg/kg) was compared with groups IV to VII treated animals (p<0.001) [Table/Fig-4].

DISCUSSION

Metabolic syndrome has become a global epidemic. The threat of diabetic complications can be reduced substantially if properly managed [10]. The association of dyslipidaemia and heart disease is well established. Moreover, reduction of levels of harmful lipids satisfactorily decreases morbidity and mortality in Coronary Heart Disease (CHD). The concomitant treatment with glibenclamide and rosuvastatin is a rational approach in the management of diabetes and reducing associated risk factors especially in management of CVD [4]. Hence, this research project was conducted to determine the effect of rosuvastatin on BSL and IR in HFHS diet induced diabetic male albino Wistar rats.

In this study, MetS like state was induced in rats by administration of HFHS prepared with mixture of vegetable oil with coconut oil. These ingredients are commonly used by a large population to cook food, which could be responsible for developing hyperlipidaemia. High sugar diet was given to rats as 25% fructose. As compared to glucose, dietary fructose is stronger inducer of insulin resistance, impairment of glucose tolerance, high insulin levels, abnormal lipid levels, hypertriglyceridaemia, and increased blood pressure [11]. Animal model with hyperlipidaemic and insulin resistant states that imitated the pathogenesis of clinical effects as observed in patients as a result of imbalanced diet intake was developed by feeding HFHS diet to rats [12]. In this study, animal model was developed by feeding HFHS diet for 8 weeks to rats, which showed a significant rise in their injurious serum lipid and lowering of HDL. Additionally, high Fasting Blood Glucose (FBG), insulin and HOMA-IR values were also observed to be representing a MetS.

Effect on blood sugar levels: The HFHS (DC) group has demonstrated a rise in the FBG. There was a significant rise in FBG at the end of 8th week in comparison with control group was the proof of induced hyperglycaemia as in MetS. All the treated groups demonstrated a decline in BSL indicating their blood glucose lowering action.

There was significant rise in blood glucose level in all treated groups up to four weeks when compared with control group. This study results are in congruence with results of Munshi RP et al., indicating that we could achieve the development of diabetic rat model by feeding HFHS [12].

	Insulin level		HOMA-IR	
Treatment (mg/kg)	Week 4	Week 8	Week 4	Week 8
Control (Distilled water)	4.5±0.6	4.6±0.9	1.1±0.2	1.0±0.9
Diabetic control (HFHS diet)	17.1±0.5***	21.3±0.9***	5.5±2.2***	7.8±0.7***
G (5 mg/kg) + HFHS	18.1±0.9***	17.4±0.9***###	5.8±0.3***	5.3±0.3***###
R (5 mg/kg) + HFHS	18.5±0.4***	14.5±0.6***###$$$	6.3±0.2***###$	3.9±0.3***###$$$
R (10 mg/kg) + HFHS	18.3±0.6***	11.2±0.2***###$$$	6.0±0.2***#	3.6±0.2***###$$$
G (5 mg/kg) + R (5 mg/kg) + HFHS diet	16.3±1.5***$$	13.2±1.7***###$$$	5.3±0.6***	3.6±0.6***###$$$
G (5 mg/kg) + R (10 mg/kg) + HFHS diet	19.0±0.8***##	10.1±0.6***###$$$	6.1±0.2***##	2.6±0.2***###$$$

[Table/Fig-4]: Effect of Rosuvastatin and Glibenclamide (alone and in combination) on Insulin level and HOMA-IR index in euglycaemic and diabetes induced male albino Wistar rats.
Rosuvastatin (R), Glibenclamide (G)
[Values expressed as Mean±SD (n=6 in each group)]
*p<0.05, **p<0.01, ***p<0.001 in comparison to vehicle treated control group
#p<0.05, ##p<0.01, ###p<0.001 as compared with HFHS diet diabetic control group
$p<0.05, $$p<0.01, $$$p<0.001 as compared with HFHS diet Glibenclamide treated group
Evaluation was carried out by one-way ANOVA which was followed with Post-Hoc Tukey's test

At the end of 8th week there was significant rise in FBG in DC animals when compared with other (groups- III, IV, and V). The FBG level increased in both the doses of rosuvastatin treated groups as monotherapy when compared to glibenclamide (5 mg/kg) treated animals ($p<0.05$). As expected there was statistical increase in FBG in glibenclamide group as compared to DC ($p<0.001$). Our results are in congruence with findings of a study conducted by Sokolovska J et al., wherein they found glibenclamide lowered the BSL and raised the insulin in similar rat models [13]. In a previous study by George AV and Augusti KT, long-term dose of glibenclamide lowered BSL, liver glycogen and protein. It also raised hepatic and serum lipids and liver organic phosphates in normal rats. Accumulation of lipids result in significant rise in weight of glibenclamide treated rats [14].

This study demonstrated that glibenclamide caused significant fall in BSL in rats with HFHS induced diabetes. Rosuvastatin did not cause any significant change of this parameter. Moreover, combination treatment was more effective for controlling BSL as compared to glibenclamide alone in rats with long-term HFHS induced diabetes. Nevertheless, more studies are required to elucidate the actual mechanism of this synergistic effect with this combination of drugs. Our results are in congruence with results of earlier studies [8,11,13]. Statins did not alter the BSL and plasma insulin induced as a result of glucose administration. There was no improvement in glucose intolerance observed during Oral Glucose Tolerance Test (OGTT) in long-term treatments of Goto-Kakizaki (GK) rats with statins in a previous literature research [15].

Statins impact glucose metabolism in several ways. They activate Endogenous Glucose Production (EGP) by up-regulation of gluconeogenic genes in hepatic cells. 16 statins upregulate the cytoplamic X receptor (PXR) which stimulates expression of proteins responsible for hepatic glucose and lipid metabolism [16,17]. However, in-vivo experimentation to assess the effectiveness of statin on glucose metabolism in T2DM patients demonstrated insignificant action on EGP. There was no change in basal EGP in type 2 diabetics in atorvastatin (10 mg for 12 weeks) or simvastatin (80 mg/day for 8 weeks) therapy [18,19].

In an experimental study conducted in Wistar rats, high BSL were produced injecting intraperitoneal low dose (25 mg/kg) streptozotocin. Rosuvastatin produced less impairment of glucose tolerance as compared to atorvastatin. Therefore, in patients at high risk with diabetes or having impaired glucose tolerance, rosuvastatin could be a better choice for preventing and treating dyslipidaemia [20]. There was no effect seen with rosuvastatin on rise in BSL and loss of body weight. However, one of the previous studies showed that treatment with pravastatin and olmesartan resulted in synergistic improvement in glucose intolerance by improving increased glucose uptake by the tissue. The effect seems to be produced by improvement in insulin sensitivity by reducing oxidative stress [21].

In patients with diabetes, statin produced a modest increase in HbA1c [22]. As a result for diabetic patients on statin should receive greater intensification of treatment. Nevertheless, statins with lower potency have been less pronounced effect on HbA1c [23].

Genetic research is required to identify the role of gene variants in the target genes for statins which could be responsible for increasing the risk of T2DM. As compared to clinical trials, population-based studies indicate relatively increased incidence of T2DM in subjects treated with statin. Emerging newer data indicates that pravastatin is the least diabetogenic among statins. Even though statins has diabetogenic potential, the consensus is in favour of use of statins as lowering cardiovascular events definitely outweigh the risk of diabetes [24,25]. As the potential for diabetogenicity varies among statins, it is advisable to personalise statin treatment by categorising patients who are at less risk of T2DM due to statins with lower diabetogenic potential [26].

Effect on insulin levels: By the end of 4 weeks, there was significant rise in serum insulin and HOMA-IR level in all groups compared to control group, whereas at the end of weeks significant decrease in serum insulin and HOMA-IR level was observed with group- [IV, V, VI, VII] treated rats ($p<0.001$), in comparison to glibenclamide (5 mg/kg). Animals which were administered glibenclamide showed group significantly reduced HOMA-IR values ($p<0.001$). All groups treated with sulfonylurea drugs showed increased insulin secretion in diabetic rats which was in congruence with earlier studies [27].

In one of the experimental studies, rosuvastatin treatment groups have increased insulin sensitivity in whole body and peripheral tissue by enhancing cellular insulin signal transduction [28]. Another study with rats models demonstrated that pravastatin and atorvastatin had paradoxical effects on insulin sensitivity and vitamin D3 levels. Pravastatin increased insulin sensitivity by elevation of 1,25-(OH)(2)-D3, whereas atorvastatin produced decrease in insulin sensitivity which was independent of 25-OH-D3 levels [29]. In another study with mice model, atorvastatin produced improvement in glucose metabolism by enhancing insulin sensitivity. Northern blot testing showed reduction in levels of mRNA of sterol regulatory element binding protein-1 (SREBP-1) and glucose-6-phosphatase (G6Pase). This may be responsible for improving glucose metabolism and insulin sensitivity [30].

Another study reveals that rosuvastatin improves insulin sensitivity in rats fed with HFD. The mechanisms involved are reduction of leptin and enhancement of Sirtuin 1, PPAR-γ and GLUT-4 expression in white adipose tissue. Sirtuin 1 is a mediator of rosuvastatin on insulin sensitivity in overweight rats induced by diet [31].

Effect on body weight: The treatment with combination of rosuvastatin and glibenclamide has significantly reduced body weight in comparison with HFHS control (DC) group than either of them as monotherapy. Similar reduction in body weight with glibenclamide was observed in a clinical study by Erqou S et al., [22].

Effect on lipid profile: By the end of 4th week significant decrease in serum TC, TG, LDL and VLDL level was observed in control group when compared to the other groups of animals. At the end of the 8th week, on comparing glibenclamide (5 mg/kg) treated group with other groups [IV, V, VI, VII], there was significant reduction in TC, LDL, VLDL, TG and increase in HDL levels which correlates with previous study results by Asad M et al., [23]. Rosuvastatin has significant pleiotropic actions which are secondary to inhibition of oxidative stress and reduction in advanced glycation end products (AGEs) accumulation. These actions may provide potential benefits apart from the lipid lowering in the management of diabetes [24].

Under different physiological conditions the factors that affect metabolism of glucose may also have action on the metabolism of lipids. It is found that cholesterol and TG levels are also increased significantly in T2DM [32,33]. Our findings suggest that rosuvastatin alone as monotherapy improved lipid profile while glibenclamide did not have any effect on lipid profile even by 8 weeks which is in congruence with results of an earlier study [34]. Previous studies have shown high levels of TG in long duration diabetic rats, because of disturbance in the TG removal mechanism. Simvastatin decreased plasma TG levels in rats both

by enhancing TG removal and by decreasing its entry into the blood [34].

Ali H et al., has described major alteration in the lipid parameters with reduction in TC, LDL VLDL and TG levels and increase in HDL on treatment with both atorvastatin and pioglitazone [10]. In our study also glibenclamide, rosuvastatin and their combination also demonstrated their property of correcting hyperlipidaemia and improving insulin sensitivity. Maximum benefit was seen with high dose rosuvastatin (10 mg/kg) as monotherapy alone followed by combination along with glibenclamide.

An experimental study in Wistar rats with streptozotocin induced hyperglycaemia revealed that hydrophilic rosuvastatin caused minor impairment in glucose tolerance. Therefore, rosuvastatin would be a preferred choice than lipophilic atorvastatin for management dyslipidaemia especially in subjects with impaired glucose tolerance or at risk of developing diabetes [20].

Limitation(s)

Chronic intake of HFHS diet produced peroxidation of lipids and abnormalities in antioxidant mechanisms in the tissues. It also causes slowing in gastric transit time, so food remains in the stomach for longer duration than required for otherwise healthy digestion. Antioxidant activities are produced by enzymatic and non-enzymatic mechanisms which were not evaluated in this study. The effects of combination therapy (glibenclamide and rosuvastatin) on the liver function parameters were not studied. High blood glucose produces increased oxidative reactions and release of free radicals along with increase in alanine transaminase and aspartate transaminase levels. By studying the effects of combination therapy (glibenclamide and rosuvastatin) on the liver function parameters more information could have been found on the hepatoprotective actions. Only two doses were studied. Duration of the study was short. Hypertension parameter was not explored which is a major criteria in MetS. No diabetic limitations were set in this study before giving treatment, but the groups treated with HFHS diet showed increase in blood glucose of diabetic level as in previous study limits.

CONCLUSION(S)

HFHS diet induced diabetes in rats is a well-accepted animal model. The combination therapy of rosuvastatin and glibenclamide demonstrated significant weight reduction, anti-hyperglycaemic and anti-lipidaemic activity which represents an effective drug combination for adequate glycaemic control and prevention of complications in the management of diabetes.

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