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RESEARCH ARTICLE

HYPOLIPIDEMIC EFFECT OF COMBINATION OF SITAGLIPTIN WITH SZYGIUM CUMINI SEEDS ON ALLOXAN INDUCED DIABETES IN RABBITS

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ABSTRACT

Diabetes is very common disease in Pakistan and abnormalities in lipid profile is one of the most common problems. Many oral hypoglycemic are available for the treatment of diabetes mellitus. This study is conducted on drug which is used for the management of diabetes that is Dipeptidylpeptidase-4 inhibitor, Sitagliptin. In this study hypolipidemic effect of combination of sitagliptin and *Szygium cumini* seed is compared with sitagliptin alone administration. The effect of drugs was observed on diabetic rabbit models. In animal models diabetes was induced by induction of alloxan. In comparision of combination of sitagliptin and *Szygium cumini* seed effects were examined on the levels of cholesterol, triglycerides and plasma lipoproteins (HDL, LDL and VLDL). It may be concluded that cholestrol HDL ratio, triglyceride, VLDL value reduced in combination as compared to sitagliptin alone in which these values are increased, that is dangerous for health. HDL reduced in sitagliptin alone but in combination, its value increased that is a good marker.

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INTRODUCTION

The DPP4 inhibitors are gliptins and these are oral hypoglycemic agents. They work by increasing the incretins that are GLP-1 and GIP (McIntosh *et al.,* 2005). This inhibits glucagon and ultimately increases insulin release. It also decreases stomach emptying in this way it decrease blood glucose levels (Richter *et al*., 2008, Herman *et al.,* 2005, Ulf *et al.,* 2008). *Szygium cumini* belong to family Myrtaceae. It is commonly known as Jambol fruit and locally as Jamun. *Syzygium cumini* is a medicinal plant seen throughout the plains from Himalayas to South India (Nadkarni, 1954). Its major constituents are glycoside called jamboline, tannins, ellagic and gallic acids. Many experimental and clinical studies conclude that different parts of the *S. cumini* especially fruits and seeds showed great activity against diabetes (Villasenor *et al*., 2000; Vikrant *et al*., 2001; Prince *et al*., 2003; Sharma *et al*., 2006) and hypolipidemic effects (Sharma *et al*., 2003; Ravi *et al.,* 2005). Sitagliptin may causes certain adverse effects as pancreatitis (Ravi *et al*., 2005; Garg *et al*., 2010; Raschi *et al*., 2013), allergic reactions, infections increase heart rate and joint pain (Giger *et al*., 3000). Diabetic patients also suffer increased risk of metabolic disturbances. S. cumini seed powder is found to reduce level of cholesterol and giving cardio protective effects. The combination of Sitagliptin and *Szygium cumini* can be helpful to reduce side effects of sitagliptin and to get better and safe effects.

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MATERIALS AND METHODS

Extract Preparation

Air-dried seeds of *S. cumini* (2.0 kg) was ground and percolated in 80% ethanol at room temperature for 15 days.

The percolate was filtered through whatman filter paper. The process was repeated for two times and the three residues obtained after filtration of the percolates were combined. Ethanol was evaporated under reduced pressure at 40°C. The crude extract obtained was lypholized and was kept for biological and pharmacological screening (Ahmad *et al*., 2012).

Animals

Rabbits of same sex of weight 1 kg were used. Each group consist of 10 animals and total 5 groups were used.

Group 1: Non diabetic control group receiving normal saline (Negative control)

Group 2: Induction of diabetes with Alloxan (Positive control)

Group 3: Induction of diabetes with Alloxan+Sitagliptin (100mg/70kg).

Group 4: Induction of diabetes with Alloxan+*Szygium cumini* (200mg/kg).

Group 5: Induction of diabetes with Alloxan+Sitagliptin (50mg/70kg) +*Szygium cumini* (100mg/kg).

Animals were housed singly with different floors (40 × 60 × 80cm) on a 12:12 hour light dark cycle in temperature (16–22°C) and humidity (30–70%). Cage racks were cleaned once per week and cage pans were cleaned thrice weekly. In the whole study, laboratory Rabbit diet and filtered tap water was given. Fresh vegetables were provided once per week. For the alloxan injection, rabbit's weights were recorded. Ketamine hydrochloride 30mg/kg and xylazine 3mg/kg was given to the rabbits to be lightly anesthetized. Normal sterile saline containing alloxan monohydrate (100mg/kg) dissolved as 5% (W/V) was administerd intravenously with 25 guage butterfly catheter for 2 minutes. Alloxan was given to non-fasted animals, food and water was given immediately to animals after recovery from anesthesia to prevent mortalities during hypoglycemic phase. After giving alloxan injection, at 4, 8, and 12 hours 10ml of 5% glucose solution was given by subcutaneous route and 20% glucose solution with help of water bottle for 2 days to prevent hypoglycemic shock when hypoglycemia confirmed (less than 70mg/dl). Automated watering system restricted during this period, to encourage intake of oral glucose solution.

Following initial alloxan injection, those rabbit whose blood glucose level maintained <180mg/dl for more than one week were administered second dose of alloxan (100mg/kg IV). During study, their blood glucose was maintain >180mg/dl. In the first 4 weeks, blood glucose has been measured using blood glucose strips once a day than once weekly thereafter in morning. when fasting blood glucose level (BGL) remains higher than 180mg/dl, than DPP4 inhibitor and *S. cumini* was administered orally in the morning for three months. For biochemical analysis, blood samples were collected at the end of experiment (Jianpu et al., 2010; Williamson *et al*., 1996; Etuk, 2010).

Biochemical Analysis: Plasma was taken for analysis by centrifugation of blood samples at 2500g for 20min. centrifugation was done at 4°C, and stored at−20°C. Cholesterol, cholesterol HDL ratio, triglycerides and plasma lipoproteins i.e. HDL, LDL and VLDL were analysed.

RESULTS

Diabetes is one of the most common disorders worldwide. As the drug therapy is be continued for a life time period so many of the studies have been conducted for the discovery of new drugs or to find out the therapeutic and toxic profile of these drugs. The current study is conducted to find the efficacy profile of combination of two hypoglycemic drugs. The combination comprise of one new class of oral hypoglycemic that is DPP4 inhibitor, Sitagliptin and one herbal remedy that is seed extract of *Szygium cumini*. Many researches proves *S. cumini* locally known as Jamun as a hypoglycemic agent that has this property in its fruit, leaves, stem bark as well as seeds. Seeds extract of *S. cumini* is also utilized in homeopaths system of medicine. The current study is to explore the hypolipidemic profile as diabetic individuals are at the risk of cardiovascular disorders. The study is conducted on diabetic animal models. The results are expressed graphically in Figures 1.1-1.3.

Effect on Cholestrol HDL ratio

The Cholestrol HDL ratio of control non-diabetic rabbits was 1 ± 0.2, while the cholestrol HDL ratio of control diabetic rabbits was 5.9 ± 0.14, which when Sitagliptin administered orally to this group reduced to 2.5± 0.20 (p=0.0001) Table 1.1).; with *S. cumini* administration cholestrol HDL ratio reduced to 4.2± 0.07 (p=0.0001) (Table 1.2); with combination of sitagliptin and *S. cumini* cholestrol HDL ratio reduced to 3± 0.19 (p=0.0001) (Table 1.3).

Effect on Cholestrol

The Cholestrol of control non-diabetic rabbits was 17±1.14 mg/dl. The Cholestrol of control diabetic rabbits was 59 ± 1.38, which when Sitagliptin administered orally to this group reduced to 46± 1.67 (p=0.0003) cholestrol level was observed. (Table 1.1); when *S. cumini* administered cholestrol level became 107± 2.13 (p=0.0001) (Table 1.2). The Cholestrol level of diabetic rabbits when Sitagliptin with *S. cumini* administered to this group was reduced to 83± 1.92 (p=0.0001) (Table 1.3).

Effect on Triglyceride

The triglycerides level of control non-diabetic rabbits (negative control group) was 110 ± 1.61mg/dl. The triglycerides of positive control group (diabetic rabbits) was 132 ± 1.38, which when Sitagliptin administered orally to this group increased to 142± 1.67 (p=0.0017) (Table 1.1); when *S. cumini* administered orally it reduced triglycerides level to 104±1.92 (p=0.0001 (Table 1.2). Sitagliptin with *S. cumini* administration reduced triglycerides level to 95± 2.17 (p=0.0001) (Table 1.3).

Effect on HDL

The HDL level of control non-diabetic rabbits (negative control group) was 17 ± 0.84 mg/dl, The HDL level of control diabetic rabbits was 10 ± 0.71, which when Sitagliptin administered orally to this group increased to 18± 1.14

Table 1.1. Effect of Sitagliptin on Diabetic and Non Diabetic Rabbit

Lipid Profile	Control (Non- diabetic)	Control Diabetic)	Treated with Sitagliptin (Diabetic)
Cholesterol HDL Ratio	1 ± 0.20	5.9 ± 0.14	2.5 ± 0.20*
Cholesterol (mg/dl)	17 ± 1.14	59 ± 1.38	46 ± 1.67*
Triglycerides (mg/dl)	110 ± 1.61	132 ± 1.38	142 ± 1.67*
HDL (mg/dl)	17 ± 0.84	10 ± 0.71	18 ± 1.14*
LDL (mg/dl)	8 ± 1.22	27 ± 1.97	30 ± 1.58
VLDL (mg/dl)	22 ± 1.14	26 ± 1.14	28 ± 2.47

Values are expressed in Mean ± SEM, n=10, * = p<0.05 when control and treated diabetic groups are compared

Table 1.2. Effect of Szygium on Diabetic and Non Diabetic Rabbit

Lipid Profile	Control (Non- diabetic)	Control Diabetic)	Treated with Szygium (Diabetic)
Cholesterol HDL Ratio	1 ± 0.20	5.9 ± 0.14	4.2 ± 0.07*
Cholesterol (mg/dl)	17 ± 1.14	59 ± 1.38	107 ± 2.13*
Triglycerides (mg/dl)	110 ± 1.61	132 ± 1.38	104 ± 1.92*
HDL (mg/dl)	17 ± 0.84	10 ± 0.71	25 ± 1.64*
LDL (mg/dl)	8 ± 1.22	27 ± 1.97	97 ± 1.64*
VLDL (mg/dl)	22 ± 1.14	26 ± 1.14	20 ± 1.22*

Values are expressed in Mean ± SEM, n=10, * = p<0.05 when control and treated diabetic groups are compared

Table 1.3. Effect of Combination (Sitagliptin + Szygium) on Non Diabetic and Diabetic Rabbit

Lipid Profile	Control (Non Diabetic)	Control (Diabetic)	Treated with Combination (Diabetic)
Cholesterol HDL Ratio	1 ± 0.20	5.9 ± 0.14	3 ± 0.19*
Cholesterol	17 ± 1.14	59 ± 1.38	83 ± 1.92*
Triglycerides	110 ± 1.61	132 ± 1.38	95 ± 2.17*
HDL	17 ± 0.84	10 ± 0.71	27 ± 1.97*
LDL	8 ± 1.22	27 ± 1.97	73 ± 1.14*
VLDL	22 ± 1.14	26 ± 1.14	19 ± 1.12*

Values are expressed in Mean ± SEM, n=10, * = p<0.05 when control and treated diabetic groups are compared

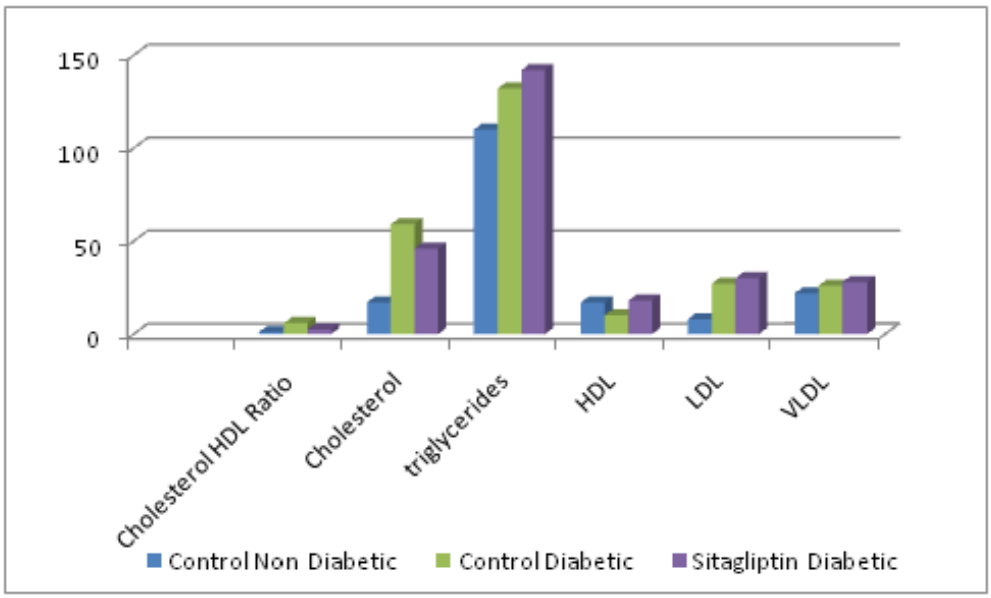

Figure 1.1: Effect of Sitagliptin on lipid profile in Diabetic Rabbits

(p=0.0003) (Table 1.1). *S. cumini* administration increased HDL level to 25± 1.64 (p=0.0001) (Table 1.2); while combination that is Sitagliptin with *S. cumini* administration increased HDL level to 27± 1.97 (p=0.0001) (Table 1.3).

Effect on LDL: The LDL level of negative control group was 8 ± 1.22 mg/dl. The LDL level of control diabetic rabbits (positive control group) was 27± 1.97, which when Sitagliptin administered to diabetic group increased upto 30± 1.58 (p=0.2697 (Table 1.1). *S. cumini* administration LDL level increased to 97± 1.64 (p=0.0001) (Table 1.2). when combination of Sitagliptin and *S. cumini* administered to diabetic group LDL level became 73± 1.14 (p=0.0001) (Table 1.3).

Effect on VLDL

The VLDL level of control non-diabetic rabbits was 22± 1.14mg/dl. The VLDL level of control diabetic rabbits was 26 ± 1.14, which when Sitagliptin administered to diabetic group increased to 28± 2.47 (p=0.4832) (Table 1.1).

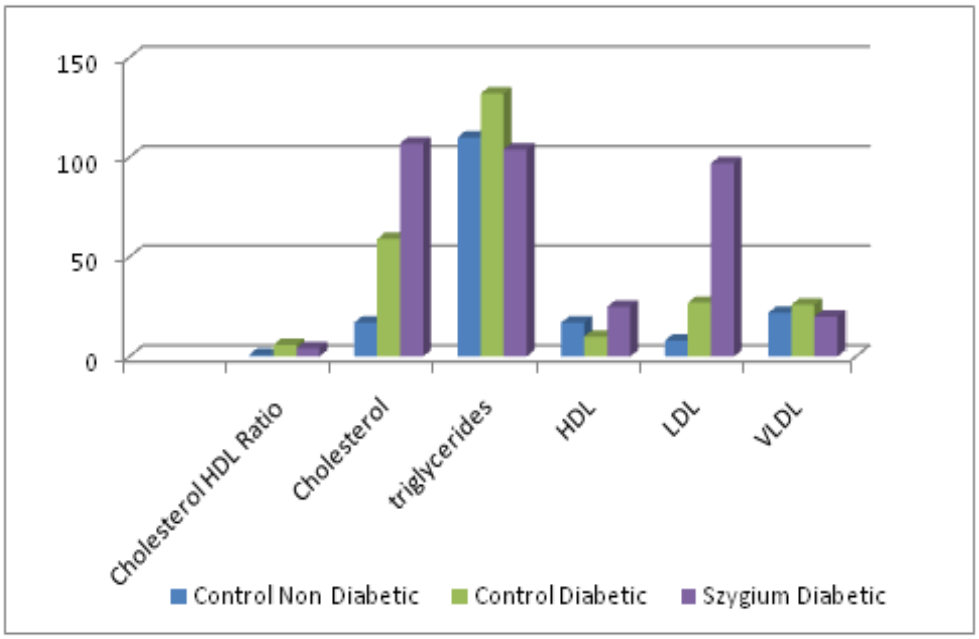

Figure 1.2: Effect of *S. cumini* on lipid profile in Diabetic Rabbits

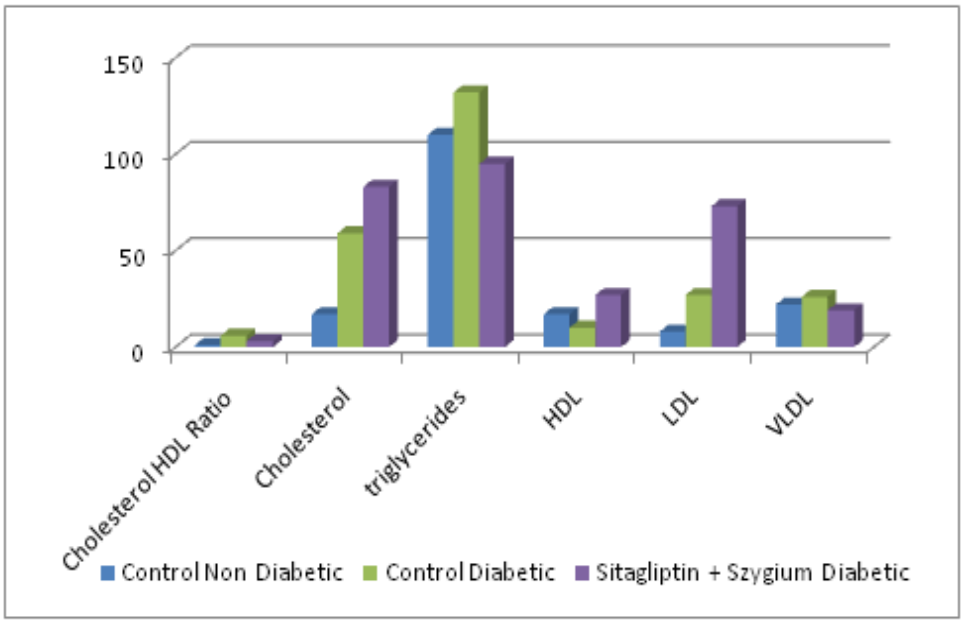

Figure 1.3. Effect of Sitagliptin with *S. cumini* on lipid profile in Diabetic Rabbits

By the *S. cumini* administration VLDL level decreased to 20± 1.22 (p=0.0071) (Table 1.2). Sitagliptin with *S. cumini* administration reduced VLDL level to 19± 1.12 (p=0.0001) (Table 1.3).

DISCUSSION

Effect of Sitagliptin alone and in combination of *S. cumini* are observed on lipid profile of diabetic rabbit models. Cholestrol HDL ratio, triglycerides and VLDL are decreasing by combination similarly HDL is increasing. Sitagliptin alone is reducing cholesterol while combination is increasing it. The level of LDL is increasing with the administration of either alone drugs or in combination. When Sitagliptin alone was given to diabetic group, effects on lipid profile are as follows i.e. triglycerides, LDL, VLDL and HDL level were increased.

When control diabetic compared with sitagliptin diabetic, p-value is significant in cholesterol (0.0003), HDL (0.0017) and triglyceride (0.0003), while insignificant in LDL (0.2697) and VLDL (0.4832). Cholestrol HDL ratio reduced in diabetic group, p-value (0.0001) is significant in it. But significant difference due to increase of all p-values is not good for health except HDL. Sindra *et al*., in 2016 worked on dried seed powder of *S. cumini* on patients and reported hypolipidemic effects (Sindra *et al*., in 2016). In present study when Szygium alone was given, in diabetic group cholestrol HDL ratio, triglyceride, VLDL levels reduced, while HDL level, cholesterol and LDL levels increased. While when combination of Sitagliptin and *S. cumini* was given in diabetic group, cholestrol HDL ratio, triglyceride, VLDL value reduced that is good for health. HDL, LDL and cholestrol value increased. As HDL level increased in combination which is good. p-value (0.0001) gives significant difference in all

diabetic groups taking combination as compared to Sitagliptin alone in which p-value is insignificant. So there is a difference between Sitagliptin alone and in combination. On comparison of Sitagliptin alone in comparision to combination of sitagliptin and *Szygium cumini* in diabetic group, p-value is significant in all. On comparison of Szygium alone in comparision to combination in diabetic group, p-value is significant in all except HDL. Comparing the results cholesterol and LDL levels are increasing while triglycerides and VLDL levels are reducing also increasing levels of HDL are found that is good sign that can be helpful in reducing cardiovascular risk.

Conclusion

Sitagliptin has different effects on various functions of body in diabetic animals. Effects are good in combination of sitagliptin with szygium, as sitagliptin dose is reduced to about half in combination but reduction of blood sugar is more than sitagliptin alone. In lipid profile, cholesterol HDL ratio, triglyceride and VLDL value reduced in combination as compared to sitagliptin alone in which these values increased, that is dangerous for health. HDL reduced in sitagliptin alone but in combination, its value increased that is a good marker.

Conflict of Interect Statement: We declare that we have no conflict of interest.

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