IJBPAS, July, 2018, 7(7): 1419-1431

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EFFECT OF ANTIDIABETIC DRUG SITAGLIPTIN WITH SZYGIUM CUMINI (L.) SKEELS ON HEPATIC AND RENAL ACTIVITY IN RABBITS

JAHAN N[*1], KHAN S[2], MEHJABEEN[3], AHMAD M[4] AND OWAIS F[5]

1: Chairperson Department of Pharmacology, Department of Pharmacology, Dow College of Pharmacy, Karachi, Pakistan

2: Senior Lecturer Department of Pharmacology, Department of Pharmacology, United Medical and Dental College, Karachi, Pakistan

3: Associate Professor, Department of Pharmacology, Federal Urdu University of Arts Science and Technology, Karachi, Pakistan

4: Professor, Department of Pharmacognosy, Federal Urdu University of Arts Science and Technology, Karachi, Pakistan

5: Lecturer, Department of Pharmacology, Federal Urdu University of Arts Science and Technology, Karachi, Pakistan

***Corresponding Author: E Mail: noor.jahan@duhs.edu.pk; Mob.: 03330218226**

Received 4th March 2018; Revised 25th March. 2018; Accepted 25th April 2018; Available online 1st July 2018

https://doi.org/10.31032/IJBPAS/2018/7.7.4495

ABSTRACT

Diabetes is one of the disorders that require life time management. Oral hypoglycemic are widely utilized by diabetic individuals to control sugar level. An abnormality in liver function markers is one of the major problems and certain drug treatments may be one of its reason. Diabetic nephropathy is also one of the most common complication of diabetes melitus. The purpose of this study is to assess the effect of antidiabetic drugs DPP-4 inhibitors as sitagliptin and *Szygium cumini* seeds on liver and kidney function. In this study, hepatic and renal effect of combination of sitagliptin and *Szygium cumini* seeds are compared with sitagliptin alone. Effects were examined on the levels of bilirubin, alkaline phosphatase, Gamma GT and SGPT for the

liver function. The effects were also observed on urea, creatinine, phosphorus, uric acid and calcium level. It may be concluded that in liver function test, there is a difference between sitagliptin alone and in combination with *S. cumini* because in combination mainly in diabetic animals, values are near to normal limit as compared to sitagliptin alone in which values are remarkably changed as decreased level of SGPT. Similarly effect of combination is good for kidney function markers as toxicity is reduced and kidney function markers not change remarkably in combination. But in sitagliptin alone, urea and phosphorus reduced too much and uric acid remarkably increased.

Keywords: DPP-4 inhibitors, Sitagliptin, *Szygium cumini*, antidiabetic, liver function markers, Kidney function, diabetic nephropathy

INTRODUCTION

Oral hypoglycemics are effective for the treatment of type II diabetes mellitus. Certain classes of oral hypoglycemic are utilized as secretagogues and sensitizers. Certain new incretin analogues as gliptins (DPP4 inhibitors) are also widely utilizing drugs now a days for treatment of diabetes [1-3]. Gliptins decreases stomach emptying they also control sugar level by increasing the level of insulin [4]. Many herbal drugs are also effective for diabetes. Herbal drugs can also be utilized in combination with allopathic medication. *S. cumini* is also known as Jamun and it fruits, seeds, leaves and stem bark all are reported to have antidiabetic activity. Previous studies report that combination of Sitagliptin and *S. cumini* gives better control of sugar level in diabetic animal models. Family of *Szygium cumini* is Myrtaceae and it is widely distributed in regions of Himalayas to South India [5]. The plant is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. The seeds are claimed to contain alkaloid, jambosine, and glycoside jambolin or antimellin, which halts the diastatic conversion of starch into sugar [6-7]. Medicinal plants having flavonoids have hepatoprotective potential [8]. There was need to screen effect of combination of these two drugs on hepatic and renal function.

MATERIAL AND METHOD

Extract Preparation:

The extraction was done by using the method of Ahmad *et al*, 2012. Air-dried seeds of *S. cumini* (2.0 kg) were used for the activity. Thick ethanolic extract was obtained that was air dried and dilutions were made in distilled water for oral administration to rabbits [9].

Animals:

Rabbit of same sex of weight 1 kg were used. Group 1: Non diabetic control group receiving normal saline; Group 2: Induction of diabetes with Alloxan (Diabetic 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).

Induction of Diabetes:

Diabetes was induced by using alloxan. Method of Jianpu was used for alloxan injection, rabbit's weights were recorded [10]. 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 administer intravenously with 25 guage butterfly catheter for 2 minutes. The animals having fasting blood glucose level higher than 180mg/dl, 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 for liver and kidneyfunction test [11-12].

Biochemistry Analysis:

Plasma was collected by centrifugation of blood samples it 3000 rpm and 4°C for 15 minutes. Plasma was stored at−20°C. Blood samples of negative and positive control groups and treated groups were analysed. For liver function levels of Bilirubin, alkaline phosphatase, Gamma GT and SGPT levels were analysed while for kidney function urea, creatinine, calcium, uric acid and phosphorus levels were analysed.

STATISTICAL ANALYSIS:

Data was analysed by one way ANOVA using SPSS version 21. Tukey's post-hoc test was used. Results are expressed in Mean±SEM taking significance $p<0.01$ and $p<0.001$.

RESULTS

Effect on liver function:

The total bilirubin level of control non-diabetic rabbits was 0.05 ± 0.01 mg/dl. The total bilirubin level of control diabetic was 0.13 ± 0.03, which when Sitagliptin, sygium and combination administered orally to the group became 0.05±0.02, 0.06±0.02 and 0.05±0.01.respectively. There was significant decrease in total bilirubin. In direct bilirubin level there was no significant change (Table 1; Graph 1.1, 1.3 and 1.5).

The SGPT level of control non-diabetic rabbits was 51 ± 1.91 U/L. The SGPT level of control diabetic was 14 ± 1.50, which

when Sitagliptin administered orally to this group it reduced to 2± 0.58. When sygium administered it became 31 ± 1.16 while with combination SGPT value raised to 63 ± 3.77 (Table 1).

The alkaline phosphatase level of control non-diabetic rabbits was 53 ± 1.36 U/L. The alkaline phosphatase level of control diabetic group was 20 ± 1.72. When sitagliptin, sygium and combination was administered it increased it to 32 ± 2.37, 62 ± 3.21 and 47 ± 2.73 respectively (Table 1).

The GAMMA GT level of control non-diabetic rabbits was 13 ± 1.03 U/L, which did not show significant elevation when Sitagliptin was administered. While with szygium and combination it increased upto 18± 1.83 and 20 ± 1.59 respectively (Table 1; Graph 1.2, 1.4 and 1.6).

Effect on Kidney Function:

The urea level of control non-diabetic rabbits was 41 ± 1.61mg/dl. The urea level of control diabetic group was 42± 1.58. When Sitagliptin and Sygium was administered alone to animal groups it reduced to 28± 1.30 and 31 ± 1.00 respectively. With the administration of combination it showed no significant change that is 41 ± 2.12 (Table 2; Graph 2.1-2.3).

The creatinine level of control non-diabetic rabbits was 1.47 ± 0.02 mg/dl. The creatinine level of control diabetic rabbits was 0.79 ± 0.02. When Sitagliptin, Sygium and combination was administered creatinine increased to 0.89± 0.01, 1.06 ± 0.02 and 0.83 ± 0.01 respectively (Table 2). There was no significant change in the level of calcium with the administration of Sitagliptin, Szygium and combination in comparision to diabetic control group that is 13.06 ± 0.03.

The uric acid of control non-diabetic rabbits was 1.07 ± 0.01 mg/dl. The uric acid of control diabetic rabbits was 1.1 ± 0.03. When Sitagliptin, szygium and combination was administered it increased to 5.37 ± 0.21, 7.42 ± 0.19 and 3.19 ± 0.06 respectively (Table 2).

The phosphorus level of control non-diabetic rabbits was 9.1 ± 0.27 mg/dl. The phosphorus level of control diabetic rabbits was 4.82 ± 0.16. When Sitagliptin, szygium and combination administered it reduced phosphorous level to 0.79±0.19, 1.31 ±0.02 and 3.82 ± 1.24 respectively (Table 2; Graph 2.1-2.3).

Table 1: Effect of Sitagliptin, *S. cumini* and combination on hepatic activity in Diabetic Rabbits

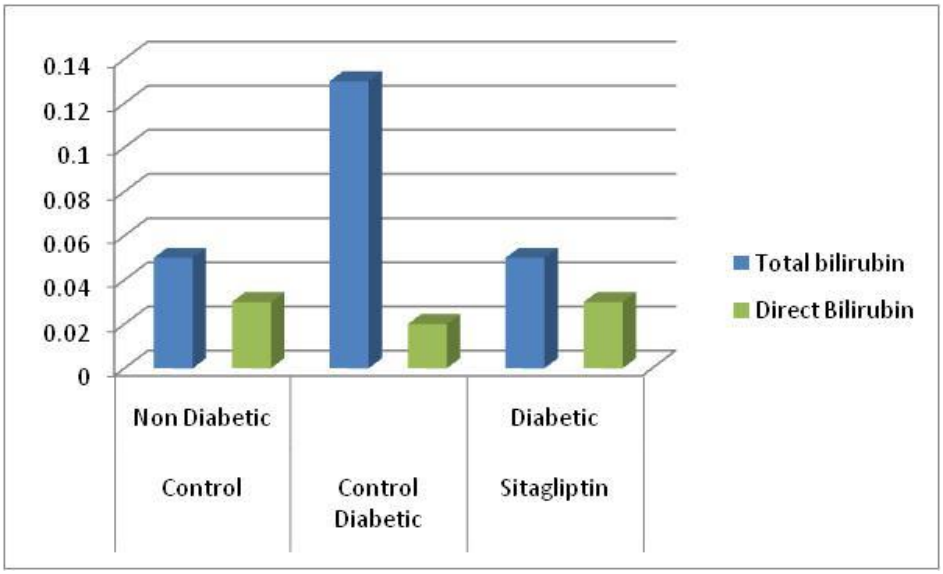
	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	SGPT (U/L)	Alkaline Phosphatase (U/L)	Gamma GT (U/L)
Normal control	0.05± 0.01	0.03± 0.01	51±1.91	53± 1.36	13± 1.03
Diabetic Control	0.13± 0.03	0.02± 0.01	14± 1.50	20± 1.72	11± 0.51
Treated 1 (Sitagliptin)	0.05 ± 0.02*	0.03 ± 0.02	2 ± 0.58**	32 ± 2.37*	13± 0.92
Treated 2 (*S. cumini*)	0.06 ± 0.02*	0.03 ± 0.01	31 ± 1.16*	62 ± 3.21**	18± 1.83*
Treated 3 (Sitagliptin + *S. cumini*)	0.05± 0.01*	0.03± 0.02	63 ± 3.77**	47 ± 2.73**	20 ± 1.59*

Values are expressed in Mean ± SEM, n=10, Results of diabetic treated groups are compared with diabetic control group; *= p<0.01;= p<0.001**

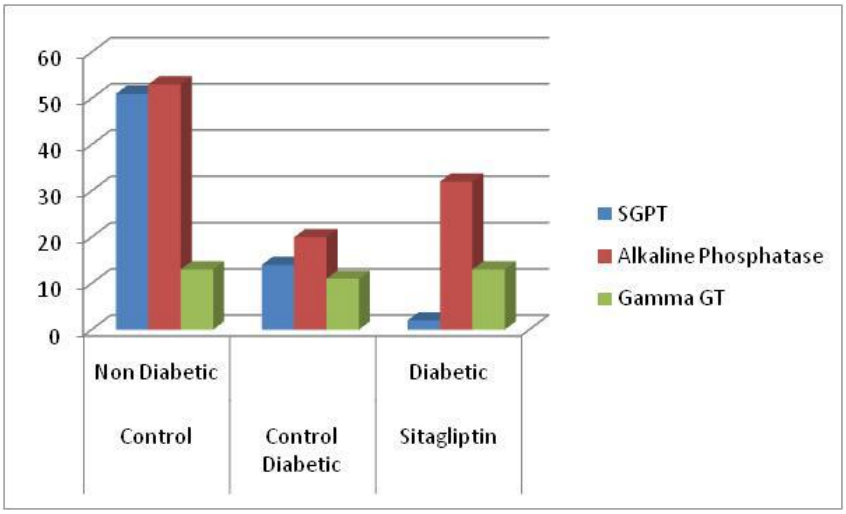
Table 2: Effect of Sitagliptin, S. cumini and combination on renal activity in Diabetic Rabbits

	Urea (mg/dl)	Creatinine (mg/dl)	Calcium (mg/dl)	Uric Acid (mg/dl)	Phosphorus (mg/dl)
Normal control	41 ± 1.61	1.47 ± 0.02	13.71 ± 0.10	1.07 ±0.01	9.1 ±0.27
Diabetic Control	42 ± 1.58	0.79 ± 0.02	13.06 ± 0.03	1.1 ± 0.03	4.82 ± 0.16
Treated 1 (Sitagliptin)	28 ± 1.30**	0.89 ± 0.01*	13.71 ± 0.06	5.37 ± 0.21**	0.79 ± 0.19**
Treated 2 (S.cumini)	31 ± 1.00**	1.06 ± 0.02**	14.17 ± 0.03	7.42 ± 0.19**	1.31 ±0.02**
Treated 3 (Sitagliptin + S. cumini)	41 ± 2.12	0.83 ± 0.01	13.16 ± 0.03	3.19 ± 0.06**	3.82 ± 1.24*

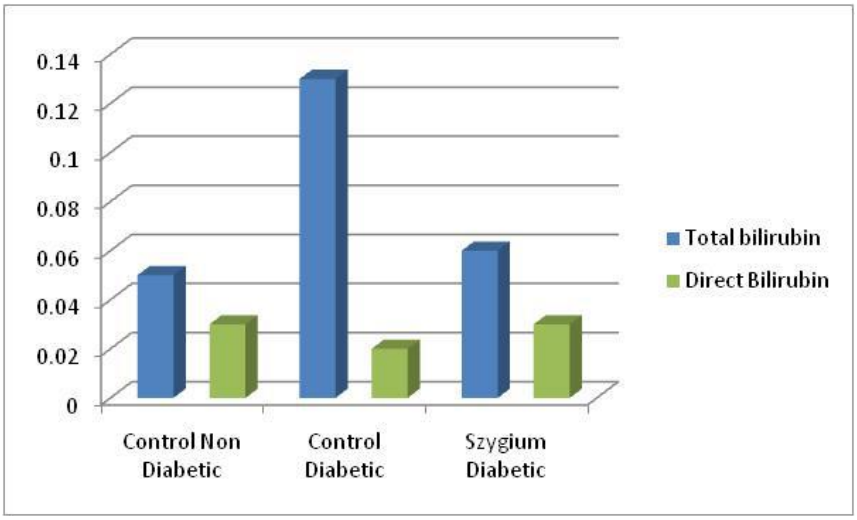
Values are expressed in Mean ± SEM, n=10, Results of diabetic treated groups are compared with diabetic control group; *= p<0.01;= p<0.001**



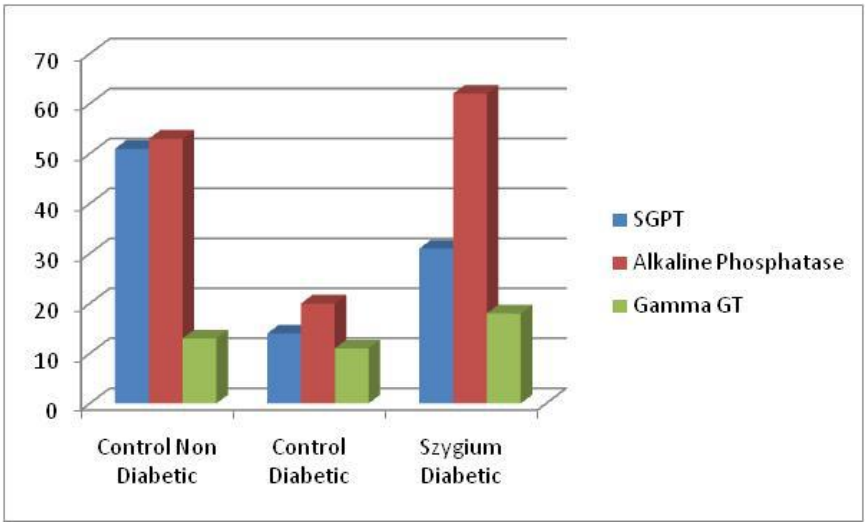
Graph 1.1: Effect of Sitagliptin on total and direct bilirubin in Diabetic Rabbit



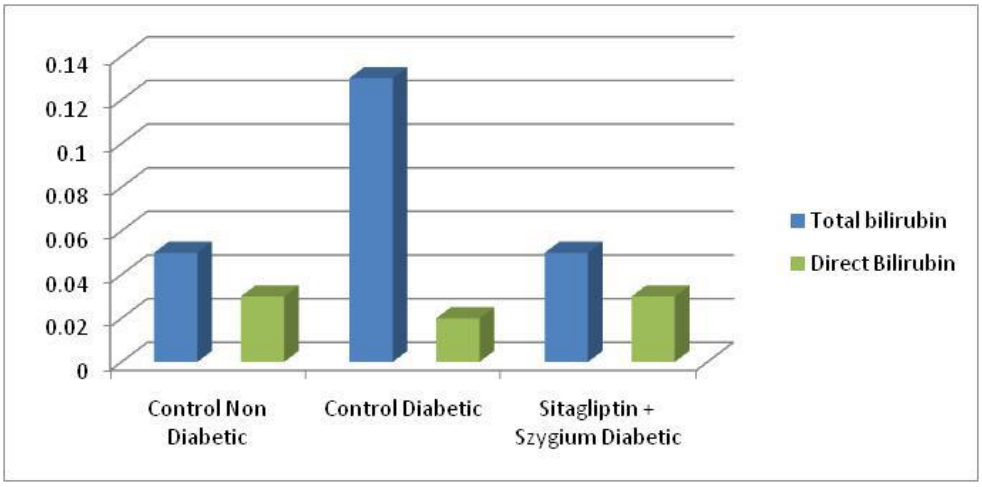
Graph 1.2: Effect of Sitagliptin on liver enzymes in Diabetic Rabbit



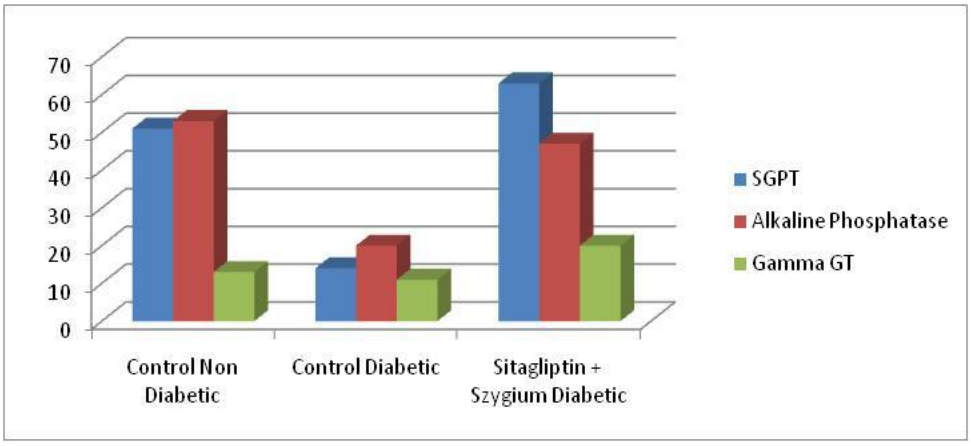
Graph 1.3: Effect of Szygium on total and direct bilirubin in Diabetic Rabbit



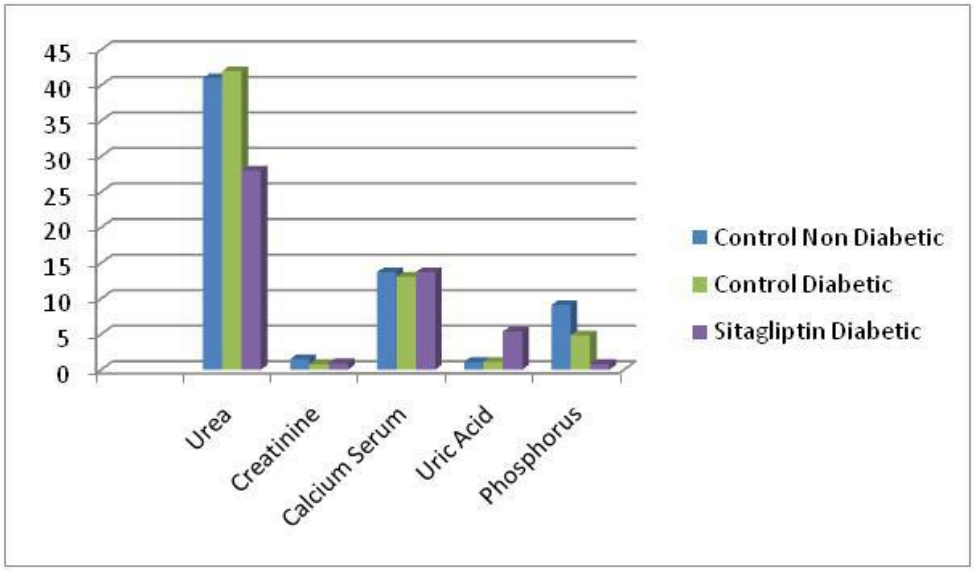
Graph 1.4: Effect of Szygium on liver enzymes in Diabetic Rabbit



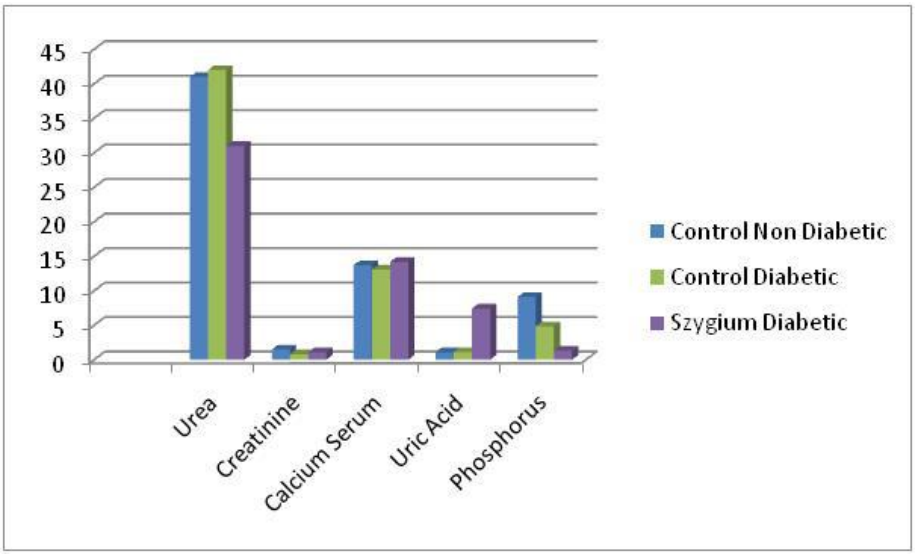
Graph 1.5: Effect of Sitagliptin with Szygium on total and direct bilirubin in Diabetic Rabbit



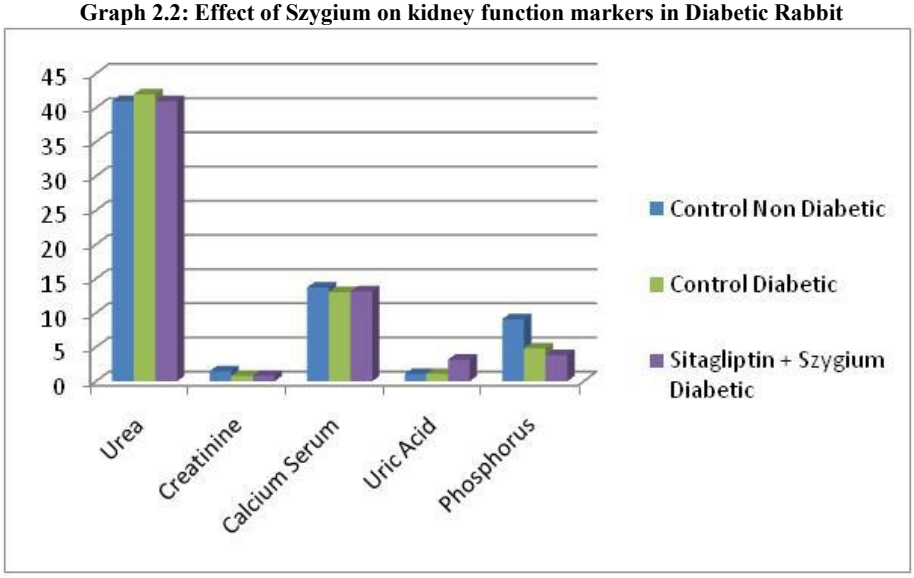
Graph 1.6: Effect of Sitagliptin with Szygium on liver enzymes in Diabetic Rabbit



Graph 2.1: Effect of Sitagliptin on kidney function markers in Diabetic Rabbit



Graph 2.2: Effect of Szygium on kidney function markers in Diabetic Rabbit



Graph 2.3: Effect of Sitagliptin with Szygium on kidney function markers in Diabetic Rabbit

DISCUSSION

The antidiabetic drugs sitagliptin and *S. cumini* were used as combination on animal models and hepatic and renal effects were observed on alloxan induced rabbits. Sitagliptin alone was given to diabetic and non diabetic groups, effects on liver function markers were observed i.e. alkaline phosphatase and Gamma GT levels were increased while SGPT level was remarkably decreased while with szygium and combination SGPT level was increased. No significant effect on bilirubin level was observed. Abo-Haded et al., worked on hepatoprotective effect of sitagliptin by methotrexate induced hepatotoxicity and it was reported that serum glucose level was not significantly altered among different

experimental groups. Methotrexate treatment increased the serum activities of ALT, AST, ALP and LDH compared to the control group. Pretreatment with sitagliptin caused a significant decrease in liver transaminases, ALP and LDH levels compared to Methotrexate group in a dose dependent manner. It was also reported that sitagliptin pretreatment alleviated markers of oxidative stress and improved the antioxidant capacity of the liver also sitagliptin pretreatment up-regulated Nrf2 mRNA and restored Nrf2 binding activity compared to Methotrexate group. Sitagliptin restored normal levels of inflammatory cytokines (nitrite/nitrate, TNF-α, IL-1β, IL-6) [13].

Shrotri et al, investigated hypoglycemic properties of *Eugenia jambolana* [14]. Seed extract contain certain constituents that can give hepatoprotection due to its antioxidant potential. The seeds have reported to be rich in flavonoids, a well-known antioxidant, which accounts for the scavenging of free radicals and protective effect on antioxidant enzymes and also found to have high total phenolics with significant antioxidant activity [15-16]. When *S. cumini* seed extract was given to assay hepatic activity, alkaline phosphatase, SGPT and Gamma GT values increased while there was no effect on direct bilirubin level. The level of total bilirubin was reduced but the result was not highly significant. When combination of sitagliptin and *Szygium cumini* was given, alkaline phosphatase and Gamma GT and SGPT levels increased. Direct bilirubin was not significantly altered.

Sitagliptin reduced levels of SGPT that can be elevated by the combination of sitagliptin and *S. cumini*. Similarly level of alkaline phosphatase much increased with the *S. cumini* that can reduce by the combination of Sitagliptin and *S. cumini.*

In present study effect of Sitagliptin and *S. cumini* on renal parameters was also observed. When Sitagliptin alone was given to control non diabetic and diabetic groups; levels of kidney function markers as urea and phosphorus remarkably reduced and uric acid and creatinine levels were increased. Sitagliptin effect on kidney markers show increase in uric acid and creatinine levels while level of urea and phosphorus was reduced and calcium levels were not altered. Jingjing et al., in 2014 demonstrate that sitagliptin treatment is effective in attenuating dyslipidemia-related renal injury. This protective effect may be associated with the activation of AMPK and the inhibition of Akt, TGF-βl, and FN, as well as the p38/ERK MAPK signaling pathway. Additionally, this protective effect of

sitagliptin appears to be distinct from its beneficial effects on glucose and lipid metabolism, and may account for enhanced GLP-1 levels [17].

Chaudhuri et al., reported anti-inflammatory activity of *S. cumini* seed extract [18]. Shafi et al., explored antibacterial activity of *S. cumini* [19]. Due to anti-inflammatory and antibacterial activity jambolinum can be effective in certain inflammatory conditions and infections [18]. When *Szygium cumini* was administered it also increased level of creatinine and uric acid these values were increased more in comparison to sitagliptin. Phosphorus and urea level was reduced with *S. cumini* also. While level of calcium was remained unaltered. In 2014 Maiti et al., also worked on *S. cumini* activity on streptozotocin indeced diabetic rat metabolic disorders they found that levels of glycogen in liver and skeletal muscle tissues were recovered by treatment with *S. jambolanum* in diabetic rats as compared with the untreated diabetic rats. Levels of serum urea, uric acid and creatinine were increased in diabetic rats significantly as compared with the control group, which were resettled in the control group after treatment with mother tincture of *S. jambolanum* in diabetic animals. Alongside, significant recovery in the activities of antioxidant enzymes like catalyse, peroxidase and super oxide dismutase, the levels of free radicals generated as bye products in hepatic and renal tissues were also observed in the treatment with mother tincture of *S. jambolanum* treated diabetic animals with respect to the untreated diabetic animals [20].

When combination of Sitagliptin and *S. cumini* was given, urea, phosphorous slightly reduced and creatinine, uric acid slightly increased. p-value is significantly changed in all except phosphorus in diabetic group.

So there is a difference between Sitagliptin alone and in combination with *S. cumini* as urea and phosphorus reduced too much in Sitagliptin as compared to combination in which they slightly reduced. Uric acid remarkably increased in group treated by Sitagliptin as compared to combination in which too much change not occurred.

CONCLUSION

Hence in liver function test, in combination mainly values are near to normal limit as compared to sitagliptin alone in which values are remarkably changed i.e decreased as in SGPT. Similarly level of alkaline phosphatase is much raised with *S. cumini* alone while in combination it is reduced. When we compare the effect on kidney function markers we can conclude that

combination is good for kidney function markers and kidney function markers not change remarkably in combination. But in sitagliptin and *S. cumini* alone, urea and phosphorus reduced too much and uric acid remarkably increased.

REFRENCES

[1] Martin C. S. Wong, Harry H. X. Wang, Mandy W. M. Kwan, Daisy D. X. Zhang, Kirin Q. L. Liu, Sky W. M. Chan, Carmen K. M. Fan, Brian C. Y. Fong, Shannon T. S. Li, and Sian M. Griffiths. Comparative Effectiveness of Dipeptidyl Peptidase-4 (DPP-4) Inhibitors and Human Glucagon-Like Peptide-1 (GLP-1) Analogue as Add-On Therapies to Sulphonylurea among Diabetes Patients in the Asia-Pacific Region: A Systematic Review. PLoS One. 2014; 9(3): e90963. doi: 10.1371/journal.pone.0090963.

[2] Simonson DC, Kourides IA, Feinglos M, Shamoon H, Fischette CT. Efficacy, safety, and dose-response characteristics of glipizide gastrointestinal therapeutic system on glycemic control and insulin secretion in NIDDM. Results of two multicenter, randomized, placebo-controlled clinical trials. The Glipizide Gastrointestinal Therapeutic System Study Group. Diabetes Care, 1997; 20(4): 597–606.

[3] Neumiller JJ, Wood L, Campbell RK. Dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes mellitus. Pharmacotherapy. 2010; 30: 463–484.

[4] Pathak R and Bridgeman MB. Dipeptidyl Peptidase-4 (DPP-4) Inhibitors In the Management of Diabetes. P T. 2010 Sep; 35(9): 509–513.

[5] Nadkarni, K. M. *Indian Materia Medica*.1954; Vol. 1, Popular Book Depot, Bombay 7.

[6] Ayyanar M and Subash-Babu P. *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. Asian Pac J Trop Biomed. 2012 Mar; 2(3): 240–246.

[7] Charbonnel B, Karasik A, Liu J, Wu M, Meininger G. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes inadequately controlled with metformin alone.

Diabetes Care, 2006; 29(12): 2638-43.

[8] Kim JW, Kim TB, Kim HW, Park SW, Kim HP, and Sung SH. Hepatoprotective Flavonoids in *Opuntia ficus-indica* Fruits by Reducing Oxidative Stress in Primary Rat Hepatocytes. Pharmacogn Mag. 2017 Jul-Sep; 13(51): 472–476. doi: 10.4103/pm.pm_232_16.

[9] Ahmad M, Muhammad N, Mehjabeen, Jahan N, Ahmad M, Obaidullah, Qureshi M and Jan SU . Spasmolytic effects of Scrophularia nodosa extract on isolated rabbit intestine, *Pak. J. Pharm. Sci*. 2012; 25(1):267-275.

[10] Jianpu Wang, Rong Wan, Yiqun Mo, Qunwei Zhang, Leslie C. Sherwood, and SufanChien. Creating a Long-Term Diabetic Rabbit Model.Experimental Diabetes *Research.Volume*.2010; 2010: 289614.

[11] Etuk, E.U. Animals models for studying diabetes mellitus. *Agric. Biol. J. N. Am*. 2010; 1(2): 130-134.

[12] Khan S, Jahan N, Mehjabeen, Ahmad M, Asif M, Owais F. Hypolipidemic effect of combination of sitagliptin with Szygium cumini seeds on alloxan induced diabetes in rabbits. International Journal of Current Research, 2018; 10(5):69144-69148.

[13] Abo-Haded HM, Elkablawy MA, Al-johani Z, Al-ahmadi O, and El-Agamy DS. Hepatoprotective effect of sitagliptin against methotrexate induced liver toxicity. PLoS One. 2017; 12(3): e0174295.

[14] Shrotri DS, Kelkar M, Deshmukh VK, Aiman R. Investigations of the hypoglycemic properties of *Vincarosea* Cassia auriculata and *Eugenia jambolana*. Indian J Med Res. 1963; 51:464–467.

[15] Ravi K, Ramachandran B, Subramanian S. Effect of *Eugenia jambolana* seed kernel on antioxidant defense system in streptozotocin induced diabetes in rats. Life Sci.2004a; 75: 2717–2731.

[16] Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. Int J Food Sci Nutr. 2005;56:287–291.

[17] Jingjing Li, Meiping Guan, Chenzhong Li, Fuping Lyv, Yanmei Zeng, Zongji Zheng, Chengzhi

Wang and Yaoming Xue. The Dipeptidyl Peptidase-4 Inhibitor Sitagliptin Protects against Dyslipidemia-Related Kidney Injury in Apolipoprotein E Knockout Mice. Int. J. Mol. Sci. 2014; 15: 11416-11434. doi: 10.3390/ijms150711416.

[18] Chaudhuri AKN, Pal S, Gomes A, Bhattacharya S. Antiinflammatory and related actions of *Syzigium cumini* seed extract. Phytother Res. 1990; 4:5–10.

[19] Shafi PM, Rosamma MK, Jamil K, Reddy PS. Antibacterial activity of *Syzygium cumini*and *Syzygium travancoricum* leaf essential oils. Fitoterapia. 2002; 73: 414–416.

[20] Maiti S, Bera TK, Chatterjee K, Ghosh D. A study of the effect of mother tincture of *Syzygium jambolanum* on metabolic disorders of Streptozotocin induced diabetic male albino rat. Indian J of Research in Homoeopathy, 2014; 8(3): 129-135.