IJPSR (2015), Vol. 6, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 March, 2015; received in revised form, 05 May, 2015; accepted, 15 July, 2015; published 01 October, 2015

RENOPROTECTIVE EFFECT OF *OCIMUM SANCTUM* IN COMPARISON WITH OLMESARTAN MEDOXOMIL AND PITAVASTATIN IN METFORMIN TREATED DIABETIC RATS

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Keywords:

Diabetic nephropathy, Nephroprotective, *Ocimum sanctum*, Angiotensin receptor blocker, Statin

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ABSTRACT: Nephropathy is an important comorbidity associated with diabetes leading to chronic kidney disease. We studied the effect of *Ocimum sanc*tum in comparison to Olmesartanmedoxomil, Pitavastatin and their combination in prevention of this comorbidity. Male Wistar rats were given streptozotocin to induce diabetes and randomly divided into groups. Group 1 (Control group) received Metformin (94.5mg/kg/day)+Distilled Water, Group 2 received Metformin (94.5mg/kg/day) +*Ocimum sanctum* (250 mg/kg/day), Group 3 received Metformin (94.5mg/kg/day) + Olmesartanmedoxomil(1.80 mg/kg/day), Group 4 received Metformin (94.5mg/kg/day) + Pitavastatin (0.18 mg/kg/day) and Group 5Metformin (94.5mg/kg/day) + Olmesartanmedoxomil (1.80 mg/kg/day) + Pitavastatin (0.18 mg/kg/day). The effects of Metformin alone and with *Ocimum sanctum*, Olmesartanmedoxomil, Pitavastatin and their combination on blood glucose and renal function test parameters were assessed. All groups showed decrease in fasting plasma glucose and glycosylated hemoglobin. Serum urea and creatinine were increased in the Control group. However, significant improvement in all the parameters studied were seen when *Ocimum sanctum*, Olmesartan or Pitavastatin were added. Histopathological examination of kidney showed mesangial proliferation, tubular swelling, thickened glomerular basement membrane, hypertrophy in glomerular tufts andhydropic degeneration in control group and improvement in all treatment groups. The best results were seen in *Ocimum sanctum* and Olmesartan+Pitavastatin groups. Metformin alone does not prevent derangement of renal function and histological changes in the kidney in diabetes mellitus. These changes can be prevented by addition of Olmesartan and Pitavastatin or *Ocimum sanctum.*

INTRODUCTION: Diabetes mellitus (DM) is a global health crisis, which has been persistently affecting humanity, irrespective of the socioeconomic profile and geographic location of the population. As per the American Diabetes Association, diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is a complex group of disorders that disturbs the metabolism of carbohydrates, fat and protein and is characterized by increased fasting and postprandial blood sugar levels [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(10).4433-41

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(10).4433-41

It is estimated that there are currently 285 million people with diabetes worldwide and this number is set to increase to 438 million by the year 2030 [2].

Diabetics have an increased risk of developing a number of serious health problems. In almost all high-income countries, diabetes is one of the leading cause of cardiovascular disease, blindness, kidney failure, and lower limb amputation. The goal of diabetes treatment is the prevention of macrovascular complications (myocardial infarction, heart failure, ischemic stroke), as well as the microvascular complications (retinopathy, neuropathy, and nephropathy); for that reason, most patients require not only a good glycemic control but also treatment for prevention of various comorbid conditions associated with diabetes especially dyslipidemia[3]and nephropathy.

In parallel with the increase in diabetics, a dramatic increase in the prevalence of diabetic nephropathy has been seen. The renin-angiotensin system (RAS) plays an important role in the development and progression of devastating disorders in patients with diabetes [4]. In the elderly, diabetic nephropathy today accounts for no less than 46% of chronic kidney disease [5]. Approximately 40% of patients with type 1 diabetes and 5 - 15% of patients with type 2 diabetes eventually develop end stage renal disease, although the incidence is substantially higher in certain ethnic groups.

A number of synthetic drugs are available for the management of diabetes and its comorbidities. The list includes Angiotensin Converting Enzyme inhibitors (like Ramipiril, Enalapril), Angiotensin Receptor Blockers (like Olmesartan, Telmisartan and Valsartan) mainly for diabetic nephropathy. Olmesartanmedoxomil (OM) is one of the newest additions to the Angiotensin Receptor Blocker (ARB) family. Similarly, Pitavastatin is the latest addition in the pipeline for the management of dyslipidaemia. However, these synthetic drugs have been reported to have adverse effects and lack several desirable properties such as efficacy on long term use or may not be very cost effective. Moreover, the prevention of comorbidities requires administration of more than one drug leading to polypharmacy, and thereby, reducing the patient compliance [6]. Thus, searching for a new class of compounds is essential to overcome diabetic problems. Therefore, attention is being directed to the medicines of herbal origin.

One such herb is *Ocimum sanctum* (OS), the test drug of our present study, which is commonly known as Tulsi or Holy Basil. It belongs to the family Lamiaceae and has been known for its therapeutic benefits since ancient times. It is considered to be a sacred plant and is widely available across the country. Different parts of plant are used in Ayurveda and Siddha Systems of Medicine for prevention and cure of many illnesses and everyday ailments. OS is a rich source of phytochemical compounds and hence the plant exhibits innumerable pharmacological effects. This plant is known to possess antidiabetic, antimicrobial, hepatoprotective, anti-inflammatory, anticarcinogenic, neuroprotective, cardioprotective, mosquito repellent, anticoagulant, immunomodulatory effect, analgesic, antifertility, antioxidant, antimicrobial and numerous other therapeutic activities [7].

Olmesartanmedoxomil and pitavastatin (the other test drugs in our study) have shown to possess pleiotropic effects besides their main actions. With the above point in mind, this study has been planned to compare the effects of *OS* with Olmesartan and Pitavastatin alone and also in combination in Metformin treated diabetic ratsand to see whether a single herbal drug is better than the already available synthetic drugs in preventing the comorbidities in Streptozotocin induced diabetic rat model.

MATERIALS AND METHODS:

Animals:

Male albino Wistar rats weighing 150-250 grams were obtained from CPCSEA certified Animal House of Indian Institute of Toxicology Research, Lucknow. They were housed under standard laboratory conditions and fed on standard pellet diet (Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*.

All studies were performed after approval from Institutional Animal Ethics Committee (approval number: PH4/2013). Ethical guidelines for animal care and animal experimentation by CPCSEA were strictly followed.

Drugs and Chemicals:

Streptozotocin, from Sigma Aldrich, USA, was given at a dose of 40mg/kg by intraperitoneal route as per previous study [8]. Metformin manufactured by Franco-Indian Pharmaceuticals, Mumbai, was pulverized and administered orally to experimental animals in distilled water with a feeding cannula at a dose of 94.5 mg/Kg/day. Olmesartanmedoxomil manufactured by Orchid Healthcare, Chennai was administered at a dose of 1.80 mg/kg/day. Pitavastatinobtained from ZydusCadila, Mumbai, was given at a dose of 0.18mg/kg/day. All the doses of test drugs were extrapolated from human dose [9].

Fresh leaves of *Ocimum sanctum (OS)* purchased from Organic Indiawere authenticated by a botanist

at National Botanical Research Institute, Lucknow and a voucher specimen of the same was kept in department museum (sample no.OS4/13).

Preparation of Plant Extract:
The extract of *OS* was prepared as per the method of Shetty*et al* [10]. Shade dried leaves of OS were grounded and this leaf powder (50g) was mixed with 225ml of distilled water (DW) and heated for 1 hour at 75-80^{o}C on water bath. The mixture was cooled and filtered using Whattman's filter paper-no. 1.

This was repeated in 2 trials. The extract was pooled and evaporated on a hot plate at 75-80^{o}C for 1 hour, transferred in a closed glass jar and stored at 4˚C for further use. Dose of OS administered to the rats was 250mg/kg body weight dissolved in DW.

Induction of Experimental Diabetes:
Diabetes was induced by single dose of intraperitoneal injection of 40mg/kg STZ. Diabetes was allowed to develop and stabilize in these STZ-treated Wistar rats over a period of 2 days. After 2 days, diabetic rats (blood glucose above 250 mg/dl) were used for further study.

Grouping of Animals:
Animals (with blood glucose level more than 250mg/dl) were randomly divided into following groups containing 6 rats each and given following drugs in DW orally, for 8 weeks:

GROUP 1: Control- Metformin (94.5mg/kg/day)+DW

GROUP 2: Metformin (94.5mg/kg/day) + *Ocimum sanctum (*250 mg/kg/day)

GROUP 3: Metformin (94.5mg/kg/day) + Olmesar tanmedoxomil(1.80 mg/kg/day)

GROUP 4: Metformin (94.5mg/kg/day) + Pitavastatin (0.18 mg/kg/day)

GROUP 5: Metformin (94.5mg/kg/day) + Olmesar tanmedoxomil (1.80 mg/kg/day) + Pitavastatin (0.18 mg/kg/day)

Treatment was started from the 3rd day after the injection of STZ which was considered as Day 0 of experiment.

Investigations:
Rats were fasted overnight and 2 ml of blood was withdrawn in the morning from retro orbital sinus at Day 0 and 4 weeks and by cardiac puncture at 8 weeks for biochemical analysis. Fasting blood glucose and renal function (serum urea and serum creatinine) were estimated by means of fully automated autoanalyser (Erba Mannheim EM 360). Glycosylated Hemoglobin (HbA1c) was estimated in EDTA sample by ion exchange resin method.

Histopathological analysis of kidney:
The animals were sacrificed by overdose of chloroform. The right kidney was identified and dissected and stored in formalin. The kidneys were then cut in slices vertically for preparation of slides and fixed and viewed under 63X microscope (Liecadfc 425c).

Statistical Analysis:
The different groups were compared using ANOVA followed by post hoc Dunnett's T3 test. All statistical analysis was done using Statistical Package for Social Science (SPSS) 16.0 software. P value < 0.05 was considered as significant.

RESULTS:
Effect on Fasting Blood Glucose:
The effect of the test drugs have been summarized in table 1below. The baseline fasting blood glucose (FBG) was similar in all the groups (P=0.190). There was a reduction in the blood glucose levels in all the 5 groups at the end of 4 and 8 weeks with a maximum percentage reduction in the Olmesartan+Pitavastatin group (72.88%±1.65) followed by Pitavastatin group (69.19%±4.41), *Ocimum sanctum* group (68.18%±2.03), Olmesartan group (65.86%±2.65) and Control group (60.56%±4.55). The percentage decrease at 8 weeks compared to baseline was statistically significant in Olmesartan + Pitavastatin group compared to control group (P=0.005).

There were significant differences in the FBG at 4 weeks (P=0.001) among the five groups. There was also significant difference at the end of 8 weeks (P=0.000) with maximum reduction seen in the Olmesartan+Pitavastatin group (125.67 mg/dl ±4.63) and minimum reduction seen in the DW group (188.67 mg/dl ±29.68). At the end of 8

weeks, the FBG in the Olmesartan+Pitavastatin group (P=0.022) and Pitavastatin group (P=0.041) was significantly lower as compared to the Control group. FBG at 8 weeks was significantly higher than Olmesartan+Pitavastatin group in control, *Ocimum sanctum* and Olmesartan groups (P=0.022, 0.011 and 0.006 respectively). FBG in Pitavastatin group was similar to that in Olmesartan+Pitavastatin group.

TABLE 1: EFFECT OF *OCIMUM SANCTUM*, OLMESARTANMEDOXOMIL AND PITAVASTATIN ON FASTING PLASMA GLUCOSE IN METFORMIN TREATED DIABETIC RATS

GROUP	DAY 0		WEEK 4		WEEK 8		% change in 8 weeks	
	Mean (mg/dl)	95% CI	Mean (mg/dl)	95% CI	Mean (mg/dl)	95%CI	Mean (mg/dl)	95% CI
Distilled water	476.00	449.34-502.66	307.33	252.50-362.12	188.67#	157.52-219.81	-60.56#	-65.33 to -55.79
Ocimum Sanctum	444.17	414.79-473.54	238.83	204.20-273.47	141.00#	133.98-148.02	-68.18#	-70.32 to -66.04
Olmesartanmedoxomil	453.00	425.16-480.84	242.67	210.51-274.83	154.33#	142.75-165.92	-65.86#	-68.65 to -63.08
Pitavastatin	442.67	413.65-471.69	238.50	225.26-251.74	135.67*	119.88-151.45	-69.19	-73.83 to -64.56
Olmesartanmedoxomil + Pitavastatin	464.50	436.59-494.41	222.17	213.66-230.68	125.67*	120.80-130.53	-72.88*	-74.61 to -71.15
ANOVA								
F	1.662		6.587		13.830		11.525	
P	0.190		0.001		0.000		0.000	

*P<0.05 in comparison to control
#P<0.05 in comparison to Metformin + Olmesartanmedoxomil + Pitavastatin

Effect on Glycosylated Haemoglobin:

No significant difference was seen in the baseline estimation of glycosylated haemoglobin (HbA1c) (P=0.465). A reduction was seen in all the groups at 4 and 8 weeks with a maximum percentage reduction in *OS* group (19.59%±3.23) and the least reduction in Pitavastatin group (8.44%±4.74). This difference was found to be statistically significant (P=0.003). Significant difference in glycosylated haemoglobin was seen at the end of 4 weeks (P=0.005). Further significant reduction was seen at the end of 8 weeks (P=0.000). The reduction in *OS* group at the end of 8 weeks was significant in comparison to that of the Olmesartan+Pitavastatin group (P=0.024). *OS* caused a significant reduction in HbA1c after 8 weeks of treatment. No statistically significant differences compared to control group were seen in HbA1c levels in other groups. (**Table 2**)

TABLE 2: EFFECT OF *OCIMUM SANCTUM*, OLMESARTANMEDOXOMIL AND PITAVASTATIN ON GLYCOSYLATED HAEMOGLOBIN (HbA1c) IN METFORMIN TREATED DIABETIC RATS

GROUP	DAY 0		WEEK 4		WEEK 8		% change in 8 weeks	
	Mean (%)	95% CI	Mean (%)	95% CI	Mean (%)	95%CI	Mean (%)	95%CI
Distilled water	7.57	7.2-7.91	7.18	7.05-7.31	6.78	6.34-7.22	-10.28	-17.15 to -3.41
Ocimum Sanctum	7.97	7.56-8.37	7.55	7.30-7.80	6.39#	6.22-6.57	-19.59	-22.98 to-16.21
Olmesartanmedoxomil	7.78	7.36-8.21	7.27	7.06-7.47	6.29	5.94-6.65	-18.85	-26.33 to-11.37
Pitavastatin	7.73	7.29-8.18	7.19	7.01-7.36	7.06	6.85-7.29	-8.44	-13.41 to -3.46
Olmesartanmedoxomil + Pitavastatin	7.75	7.49-8.00	7.26	7.21-7.32	6.73	6.61-6.86	-13.04	-16.77 to -9.30
ANOVA								
F	0.925		4.935		7.660		5.420	
P	0.465		0.005		0.000		0.003	

*P<0.05 in comparison to control
#P<0.05 in comparison to Metformin + Olmesartanmedoxomil + Pitavastatin

Effect on Serum Urea:

The effect of the test drugs on Serum Urea have been summarised in **Table 3**. The level of Serum urea was similar on Day 0 before administration of the test drugs (P=0.544). A significant reduction in

the percentage of Serum urea was seen at the end of 8 weeks (P=0.000) with largest reduction in the Olmesartan+Pitavastatin group (52.56%±3.86) and least reduction in the Control group (14.61%±5.00). However, the *Ocimum sanctum*, Olmesartan and Pitavastatin groups also showed significant reduction in comparison to the control group (P=0.000 for each group).

At week 4, there was significant reduction in the Serum Urea (P=0.000) with maximum reduction in the Olmesartan+Pitavastatin group (54.33 mg/dl ±2.80).The Serum Urea of Olmesartan+Pitavastatin (P=0.000), Olmesartan (P=0.000), Pitavastatin (P=0.000) and *Ocimum sanctum* group (P=0.000) were significantly lower than the Control group whereas the Serum urea was significantly higher in the control group in comparison to the combination group (P=0.002).

At week 8, similar results were observed (P<0.001) and again the Serum Urea of Olmesartan+Pitavastatin (P=0.000), Olmesartan (P=0.000), Pitavastatin (P=0.000) and *Ocimum sanctum* group (P=0.000) were significantly lower than the Control group. A significant difference was seen in all the groups in comparison to the Olmesartan+Pitavastatin group (P = from 0.000 to 0.003).

TABLE 3: EFFECT OF *OCIMUM SANCTUM*, OLMESARTANMEDOXOMIL AND PITAVASTATIN ON SERUM UREA IN METFORMIN TREATED DIABETIC RATS

GROUP	DAY 0		WEEK 4		WEEK 8		% change in 8 weeks	
	Mean (mg/dl)	95% CI	Mean (mg/dl)	95% CI	Mean (mg/dl)	95%CI	Mean (%)	95%CI
Distilled water	62.11	57.15-67.09	66.58#	62.35-70.82	71.00#	68.51-73.49	14.611#	9.36 to 19.86
Ocimum Sanctum	65.50	61.59-69.41	57.95*	55.81-60.09	52.95*#	51.22-54.68	-19.04*#	-22.17 to -15.90
Olmesartanmedoxomil	64.28	60.56-68.01	54.93*	51.09-58.77	38.32*#	36.63-39.99	-40.24*#	-44.69 to -35.78
Pitavastatin	61.67	56.25-67.09	56.33*	52.66-60.01	52.02*#	47.78-56.26	-15.49*#	-20.50 to -10.47
Olmesartanmedoxomil + Pitavastatin	63.35	58.89-67.81	54.33*	51.39-57.28	30.00*	27.35-32.65	-52.56*	-56.61 to -48.50
ANOVA								
F	0.788		13.815		219.464		221.959	
P	0.544		0.000		0.000		0.000	

*P<0.05 in comparison to control

#P<0.05 in comparison to Metformin + Olmesartanmedoxomil + Pitavastatin

Effect on Serum Creatinine:

No difference was observed at baseline in the five groups (P=0.153). A reduction in the Serum Creatinine values was seen in all the groups except the control group at 4 weeks and 8 weeks. The percentage reduction ranged from 17.58%±13.01 in the control group to 59.29%± 3.31 in Olmesartan group. This difference was found to be statistically significant (P=0.000). P value was less than 0.05 in *Ocimum sanctum*, Olmesartan and combination groups in comparison to the control group.

No significant differences in Serum Creatinine was seen at Week 4 (P=0.171).However, significant difference was seen at 8 weeks (P=0.000). Highest Serum creatinine was seen in control group (1.30 mg/dl ±0.35) and lowest in Olmesartan group (0.75 mg/dl ±0.075). The decrease in Serum creatinine in *Ocimum* sanctum, Olmesartan and Pitavastatin groups was similar to that of Olmesartan +Pitavastatin. (**Table 4**)

TABLE 4: EFFECT OF *OCIMUM SANCTUM*, OLMESARTANMEDOXOMIL AND PITAVASTATIN ON SERUM CREATININE IN METFORMIN TREATED DIABETIC RATS

GROUP	DAY 0		WEEK 4		WEEK 8		% change in 8 weeks	
	Mean (mg/dl)	95% CI	Mean (mg/dl)	95% CI	Mean (mg/dl)	95%CI	Mean (%)	95%CI
Distilled water	1.58	1.22-1.94	1.12	0.85-1.39	1.30	0.94-1.66	-17.58#	-31.23 to -3.92
Ocimum Sanctum	1.74	1.63-1.85	1.12	0.99-1.24	0.89	0.82-0.97	-48.05*	-54.96 to -41.13
Olmesartanmedoxomil	1.84	1.76-1.92	0.93	0.84-1.02	0.75	0.67-0.83	-59.29*	-62.77 to -55.81
Pitavastatin	1.51	1.08-1.93	1.12	0.88-1.35	0.81	0.69-0.93	-43.30	-57.42 to -29.19
Olmesartanmedoxomil + Pitavastatin	1.77	1.68-1.85	0.95	0.78-1.12	0.77	0.68-0.85	-56.49*	-62.27 to -50.71

ANOVA				
F	1.839	1.749	10.458	18.973
P	0.153	0.171	0.000	0.000

*P<0.05 in comparison to control
#P<0.05 in comparison to Metformin + Olmesartanmedoxomil + Pitavastatin

Histopathological analysis of kidney:

The histology of Control group kidney under 63X, shows mesangial proliferation, along with tubular swelling and thickened glomerular basement membrane. Occasional glomerular tufts show hypertrophy. There is evidence of hydropic degeneration in proximal convoluted tubule and vascular congestion at places (**Fig.1A**).

However, as compared to the control group, the glomerulus of the *Ocimum sanctum* group shows normal glomeruli with normal basement membrane and no proliferation of the mesangial matrix (**Fig. 1B**). A similar picture is seen in Olmesartan Group with no glomerular hypertrophy, normal mesangial matrix and normal basement membrane. No tubular swelling is evident (**Fig.1C**). **Fig.1D** shows the examination of Pitavastatin group. The mesangial matrix is increased and renal tubules disrupted, but it is less than control group. Congestion can be seen in the blood vessels. **Fig.1E** shows almost normal renal architecture with only mild congestion.

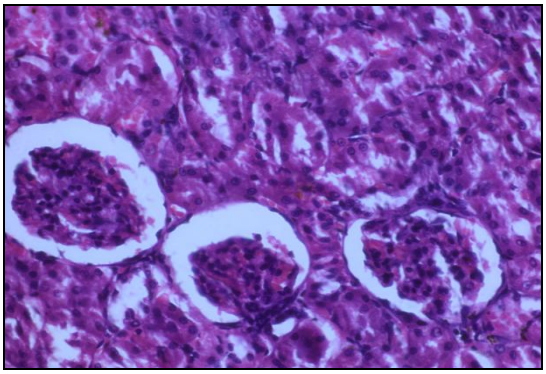

Fig. 1A

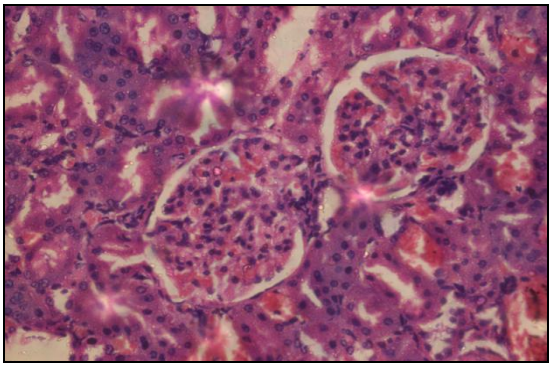

Fig. 1B

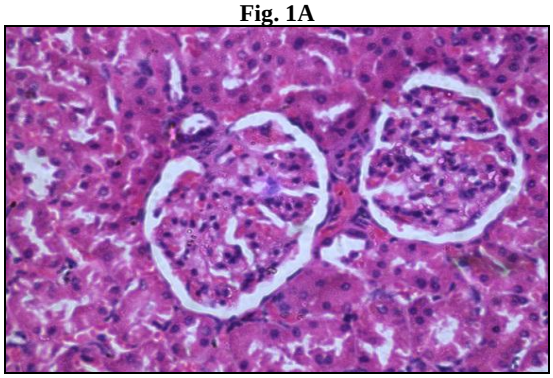

Fig.1C

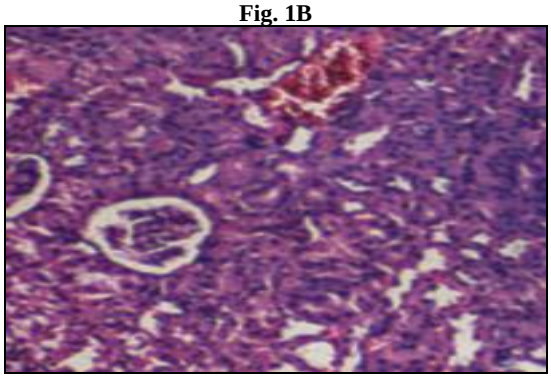

Fig. 1D

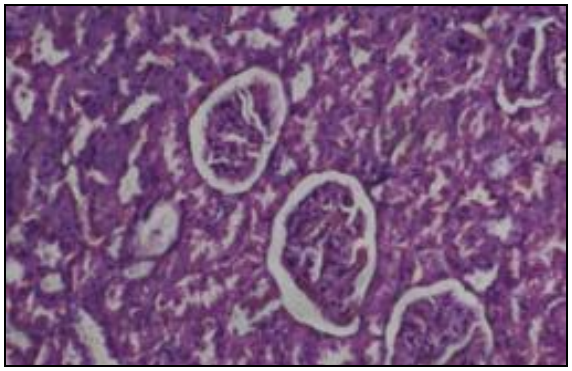

Fig. 1E

FIG.1: PICTOMICROGRAPH AT 63X OF KIDNEY ARCHITECTURE (H & E STAINING).

(**Fig 1A**- control group, 1B- *Ocimum sanctum* group, 1C- Olmesartan group, 1D- Pitavastatin group, 1E- Olmesartan + Pitavastatin group)

DISCUSSION: Present study was done to discern the effects of the herb *Ocimum sanctum* on prevention of diabetic nephropathy. The effects have been compared with Olmesartanmedoxomil, Pitavastatin and their combination.

All treatment groups showed a reduction in Fasting blood glucose as well as HbA1c. Previous studies have reported antihyperglycemic effect of *OS* [11, 12]. Blood glucose levels in rats taking Pitavastatin alone or with Olmesartan were significantly less than control group. This may be due to pleiotropic effect of Pitavastatin[13] and Olmesartan[14]. However, HbA1c levels in *OS* were significantly lower than Olmesartan+Pitavastatin group. Our results are in conformity to those of *Vijayakumar et al* [15] and *Si et al(2014)*[16] who reported significant reduction in HbA1c after one month administration of *OS* in STZ induced diabetic rats. Our study is limited by duration (8weeks) since HbA1c levels reflect blood glucose levels of previous 3 months.

Various theories have been proposed for the probable mechanisms on antihyperglycemic role of OS such as decrease in activities of carbohydrate-metabolising enzymes (hexokinase, glucose-6-phosphate dehydrogenase and glycogen synthase) [12], enhanced insulin secretion by mechanisms affecting K^+ATP channels [17] andinhibition of absorption of glucose from the intestines. *Kadian et al* [18] reported that out of ten fractions (F1-F10) isolated from hydroalcoholic extract of *OS* aerial part by column chromatography, the bioactive fraction (F5) was found to be potent antidiabetic by ameliorating glucose levels in alloxan induced diabetic rats. The spectroscopic data analysis revealed that the isolated bioactive compound elucidated was a tetracyclic triterpenoid.

Various statins have shown to increase blood sugar. However, Pitavastatin has shown to decrease blood glucose in this regard. Our results are supported by the evidence from the study done by *Soneet al* [19], in which no significant changes in FBS were seen on administering Pitavastatin in T2DM patients with hyperlipidaemia, suggesting that the influence of Pitavastatin on glycaemic status was negligible. To the best of our knowledge, no study has yet been done to study the effects of combining Olmesartan with Pitavastatin on blood glucose level and our study is the first to report decreased blood glucose with Olmesartan+Pitavastatin..

Metformin treatment alone could not prevent development of nephropathy in STZ induced diabetic rats as evidenced by an increase in serum urea levels, histopathological evidence of mesangial proliferation and thickening of glomerular basement membrane along with other changes and only a mild decrease in serum creatinine.

Addition of *OS*, Olmesartanmedoxomil or Pitavastatin to Metformin showed a dramatic decrease in serum urea levels. Similarly, significant reduction in comparison to control group was seen in serum creatinine in all the treatment groups. *Ocimum sanctum* showed a reduction similar to Olmesartan+Pitavastatin group. These findings also confirm the nephroprotective role of *Ocimum sanctum*.

OS and Olmesartan groups showed normal renal architecture which confirms their nephroprotective role. To the best of our knowledge, effect of *Ocimum sanctum* in prevention of diabetic nephropathy has not been studied previously and this is being reported for the first time.*OS* has been shown to have protective effect against mercury [20] and gentamicin [21] induced nephrotoxicity via reduction of lipid peroxidation.

We propose that OS causes renoprotection by antioxidant and anti-inflammatory mechanisms which are also shared by ARBs and statins. Diabetes induced production of AGEs may be reduced by OS due to its antioxidant effect. The phenomenon associated with diabetic nephropathy seems to be heterogeneous. They are tentatively integrated in a hypothetical scheme depicted in **Fig. 2** below, suggesting an interaction among oxidative stress, AGE formation, chronic hypoxia, iron deposition, and inflammatory cell infiltration. Abnormal iron deposition accelerates the Fenton reaction and eventual hydroxyl radical generation, which in turn increases oxidative stress and AGE formation. The last further interacts with the receptor for AGE with an attendant release of reactive oxygen species and eventual chemotactic attraction of macrophages. Chronic hypoxia in the

tubulointerstitial tissue transforms tubular cells into myofibroblasts and accelerates tissue fibrosis, which is further exacerbated by concomitant inflammatory cells infiltration, oxidative matrix protein damage, and AGE modification.

Since OS exerts antioxidative effect as reported in various studies [19], this may lead to reduction in hydroxyl radical generation and release of reactive oxygen species, AGE formation leading to protective effect of renal tubules. Further, its antinflammatory action may lead to decreased inflammatory cell infiltration leading to decreased iron deposition, hydroxyl radical generation and fibrosis.

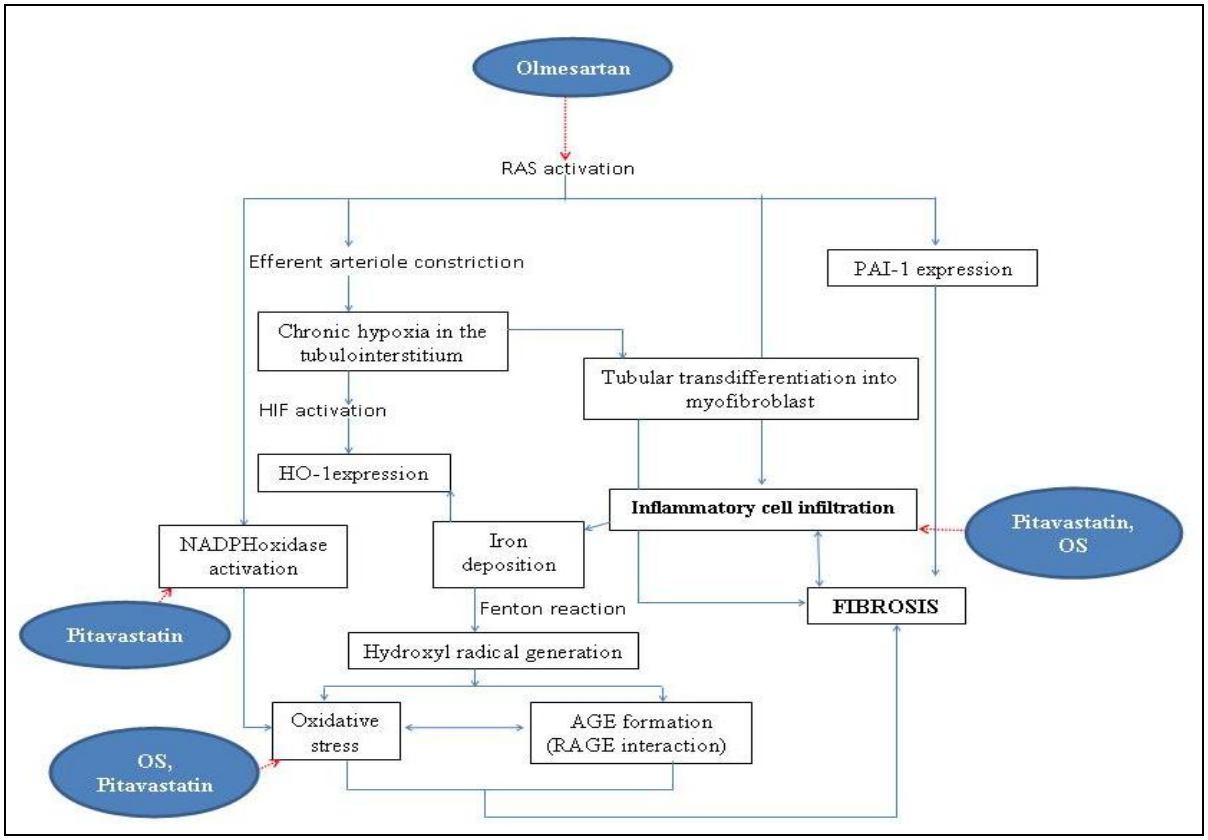

FIG.2: HYPOTHESIZED MECHANISM OF ACTION OF OS, OLMESARTAN AND PITAVASTATIN IN PREVENTION OF DN (MODIFIED FROM *INZUHARA ET AL2005*) [22] (HO-1-HEMOXYGENASE; PAI-1- PLASMINOGEN ACTIVATOR INHIBITOR; AGE- ADVANCED GLYCATION END PRODUCTS)

Similarly, in the renal architecture also Metformin alone could not prevent the changes of diabetic nephropathy as shown by the histopathological analysis. However, the addition of the test drugs improved the architecture, with greatest improvement seen in the Olmesartan+Pitavastatin and OS group.

However, we could not find any previous study that showed the effect on histopathological findings of treatment of *OS* in diabetic kidney. Moreover, to the best of our knowledge, the histopathological effect of combination of Olmesartan with Pitavastatin is being reported for the first time. Having discussed and interpreted the outcome of the present study, we can say that in most of the parameters studied, addition of *Ocimum sanctum* or combination of Olmesartan with Pitavastatin showed effective results. This shows that diabetics should not only be given only an antihyperglycemic therapy, but also add-on therapy in order to prevent the occurrence of comorbidities like diabetic nephropathy. *OS*, in this regard, proved almost equally effective, as it not only showed lowering of blood glucose and glycosylated haemoglobin levels, but also improvement in renal function test parameters. The beneficial role of this herb was also seen in the histological sections of kidney.

CONCLUSION: Our study suggests that addition of statin and an ARB in diabetics should be done to

prevent complications of diabetes like diabetic nephropathy which cannot be prevented by giving Metformin alone. Among the statins, Pitavastatin is a good choice for diabetics and prediabetics because it has no deleterious effect on glucose levels. Our study also shows that the beneficial effects such as improvement of blood glucose and nephroprotection in diabetics can also be achieved by administration of a single herbal drug, *Ocimum sanctum*. However, further studies including clinical trials are required to advocate its clinical use.

We conclude that OS given in addition to Metformin is effective in reducing blood glucose and glycosylated haemoglobin, preventing diabetic nephropathy and preventing histological changes in kidney STZ induced diabetic rats. These effects are comparable to those of combination of Olmesartan+Pitavastatin.

ACKNOWLEDGEMENT: I would like to thank organizers of national conference entitled 'Novel Tools and Treatment Approaches in Health Care System' for selecting my paper for oral presentation, organized at Faculty of Pharmacy, Integral University, Lucknow on 3rd March 2015.

REFERENCES:

1. Natarajan V, Dhas ASA. Effect of active fraction isolated from the leaf extract of Dregeavolubilis [Linn.] Benth. on plasma glucose concentration and lipid profile in streptozotocin-induced diabetic rats. Springer Plus 2013; 2:394.
2. Anjana RM, Ali MK, Pradeepa R, Deepa M, Datta M, Unnikrishnan R, Rema M, Mohan V. The need for obtaining accurate nationwide estimates of diabetes prevalence in India - Rationale for a national study on diabetes. Indian J Med Res 2011; 133: 369-80.
3. LeRoith D. Hyperglycemia, hypertension, and dyslipidemia in type 2 diabetes mellitus: goals for diabetes management. Clinical Cornerstone 2009; 9(2): S8–S16.
4. Higaki J, Baba S, Katsuya T, Katsuya T, Sato N, Ishikawa K, Mannami T, Ogata J, Ogihara T. Deletion allele of angiotensin-converting enzyme gene increases risk of essential hypertension in Japanese men. The Suita study. Circulation 2000; 101:2060–65.
5. Prakash J, Hota JK, and Singh S, Sharma OP. Clinical spectrum of chronic renal failure in the elderly: a hospital based study from eastern India. IntUrolNephrol 2006; 38(3):821-7.
6. Donath MY, Ehses JA. Type I and type II diabetes: NOD the diabetes we thought it was. ProcNatlAcadSci 2006; 103:12217–18.
7. Khan V, Najmi AK, Akhtar M, Aqil M, Mujeeb M, Pillai KK. A pharmacological appraisal of medicinal plants with antidiabetic potential. J Pharm Bioallied Sci. 2012 Jan-Mar; 4(1): 27–42.
8. Punithavathi VR, Prince PS, Kumar R, Selvakumari J. Antihyperglycaemic, antilipidperoxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic Wistar rats. Eur J Pharmacol 2011 Jan 10; 650(1):465-71.
9. Paget GE, Barnes JM. Evaluation of drug activities: Pharmacometrics. vol 1, New York. Academic Press, 1969: 154-55.
10. Shetty S, Udupa S, Udupa L. Evaluation of Antioxidant and Wound Healing Effects of Alcoholic and Aqueous Extract of Ocimum sanctum Linn in Rats. eCAM 2008;5(1)95–101.
11. Halim EM, Mukhopadhyay AK. Effect Of Ocimum Sanctum (Tulsi) And Vitamin E On Biochemical Parameters And Retinopathy In Streptozotocin Induced Diabetic Rats. Indian Journal of Clinical Biochemistry 2006; 21 (2): 181-8.
12. Narendhirakannan RT, Subramanian S, Kandaswamy M. Biochemical evaluation of antidiabetogenic properties of some commonly used Indian plants on streptozotocin-induced diabetes in experimental rats. ClinExpPharmacol Physiol. 2006 Dec; 33(12):1150-7.
13. Kawai Y, Ishida RS, Motoyama A, Kajinami K. Place of pitavastatin in the statin armamentarium: promising evidence for a role in diabetes mellitus. Drug Design, Development and Therapy 2011:5 283–97.
14. Dandan RH. Renin and Angiotensin. Goodman and Gilman's The Pharmacological Basis of Therapeutics.12th edition. China. McGraw Hill.2011.p 721-44.
15. Vijaya Kumar PR, Priya K, Srivastava P, Paul D .To Compare the Pleiotropic Effects of Telmisartan and Olmesartan in Hypertensive Patients with Metabolic Syndrome Based On ATP III Criteria.IOSR Journal Of Pharmacy. 2013; 3(1): 59-67.
16. Si X, Li P, Zhang Y, Zhang Y, Lv W, Qi D. Renoprotective Effects Of Olmesartan Medoxomil On Diabetic Nephropathy In Streptozotocin Induced Diabetes In Rats. Biomedical Reports.2014; 2: 24-8.
17. Hannan J M A, Marenah L, Ali L , Rokeya B, Flatt PR, Abdel-Wahab YHA.Ocimum sanctum leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic cells. Journal of Endocrinology. 2006; 189: 127–36.
18. Kadian R and Parle M. Therapeutic potential and phytopharmacology of tulsi. Int. J. of Pharm. & Life Sci. (IJPLS) 2012; 3(7): 1858-67.
19. Sone H, Takahashi A, Shimano H. HMG CoA reductase inhibitor decreases small dense low density lipoprotein and remnant like particle cholesterol in patients with Type 2 diabetes. Life Sci. 2002; 71: 2403-12.
20. Sharma MK, Kumar M, Kumar A Protection against mercury-induced renal damage in Swiss albino mice by Ocimum sanctum. Environ Toxicol Pharmacol. 2005 Jan; 19(1):161-7.
21. Muglikar A, ChilwantKS. Effect of *Ocimum sanctum* (OS) aqueous leaf extract on gentamicin induced nephrotoxicity in albino rats. Available from journal.mimsr.edu.in
22. Izuhara Y, Nangaku M, Inagi R, Tominaga N, Aizawa T, Kurokawa K, Strihou C, Miyata T.Renoprotective Properties of Angiotensin Receptor Blockers beyond Blood Pressure Lowering. J Am SocNephrol. 2005; 16: 3631–41.

How to cite this article:
Thadani S, Salman MT, Tewari S, Singh S, Bhagchandani D and Ahmad A: Renoprotective Effect of *Ocimum Sanctum* in Comparison with Olmesartan Medoxomil and Pitavastatin in Metformin Treated Diabetic Rats. Int J Pharm Sci Res 2015; 6(10): 4433-41.doi: 10.13040/IJPSR.0975-8232.6(10).4433-41.