ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Combined Effect Of Metformin And Sitagliptin On Oxidative Stress In Diabetic Nephropathy In Rats With Type 2 Diabetes Mellitus.

Reham E Masoud[1]*, and Amira S mohammed[2].

[1]Lecturer of Clinical Pharmacology, Portsaid University
[2]Lecturer of Clinical Pharmacology, Suez Canal University

ABSTRACT

Diabetic nephropathy is a serious complication of diabetes; we aimed to evaluate the combined renoprotective effect of metformin and Sitagliptin on rats with type 2 diabetes which was induced by high fructose for 8 weeks. Study groups included normal control, diabetic control, metformin treated, sitagliptin treated and metformin and sitagliptin treated groups, treatment continued for three months. Fasting blood sugar level, body weight, systolic blood pressure, creatinine clearance and urinary albumin creatinine ratio assessed monthly after induction of diabetes. Lipid profile and glycosated hemoglobin were assessed at the end of the study. After sacrifice, malondialdehyde and glutathione peroxidase level were assessed in kidney tissue, histopathological examination of kidney stained with H and E stain and PAS stain and intensity of lamininimmunostain were done. Study results showed that metformin and sitagliptin combination caused significant improvement of all parameters measured compared to diabetic group and groups which used one drug, there was a significant increase in antioxidant activity (decrease MDA and increase glutathione peroxidase) in kidney tissue and prevention of all pathological changes in diabetic nephropathy. We concluded that combined administration of metformin and Sitagliptin caused more significant renoprotective effect in type 2 diabetes mellitus than each drug alone.

Keywords: metformin, sitagliptin, type 2 diabetes, MDA, glutathione peroxidase

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Hyperglycemia increases oxidative stress and generate reactive oxygen species (ROS) which causes many destructive effects on body organs (1). ROS causes damage to the membranes of the cell of all body organs, causes inactivation of the antioxidant enzymes, alter ion of the endogenous genes responsible for antioxidant expression and all these effects cause onset and progression of pathology of DN (2–3). ROS causes activation of cascade of signal transduction inducing the expression profibrotic factors expression, which include fibronectin, lamin and Collagen IV, this causes extracellular matrix (ECM) accumulation, increase in inflammatory gene expression, such as IL-6 and alter gene expression for antioxidant enzymes. ROS causes activation of expression of transforming growth factor (TGF)- β1 ,combined with its down regulation of effectors connective tissue growth factor (CTGF), this leads to fibrosis of body tissues(4,5) and increases cell proliferation and ECM formation, laminin is one component of ECM, increase ECM formation is the most important pathological feature of DN (6,7).

Metformin, a well known oral hypoglycemic diguanide drug, which was used for many years in type 2 diabetes mellitus (T2DM) therapy, especially in obese patients (8). Metformin decreases most complications caused by diabetes by decreasing the level of body glucose (9); and however the precise mechanisms causing its benefits is not well understood, but it is generally accepted that metformin increases the body's sensitivity to insulin. It has been approved that metformin decreases liver gluconeogenesis and causes inhibition of absorption of sugar in the intestines (10); whereas another previous study has shown that it decreases ROS generation (11).

Sitagliptin (SIT), an inhibitor of a dipeptidyl peptidase-4 (DPP-4), causes some beneficial effects on level of glycosated hemoglobin when combined with metformin for diabetes mellitus type 2 therapy (12). other studies have shown that when use of SIT alone, it offered protection of cardiovascular system and nervous system and this may be caused by antioxidant, anti-inflammatory, and anti-apoptotic properties. It is documented that SIT may decrease renal ischemia reperfusion injury in rats (13). However, whether SIT has beneficial effect on prevention of DN remains unknown.
Therefore, in this study, we evaluate possible protective effect of metformin and sitagliptin alone and in combination on prevention of DN and possible antioxidant effects of both drugs in type 2 diabetes mellitus.

MATERIAL AND METHODS

Experimental animals:

Male wistar rats (150-160 g) were used. They were purchased from the Egyptian Organization for Biological Products and Vaccines (Egypt).Animals were kept and housed in polypropylene cages and kept in the standard laboratory environmental conditions; with free access to food and water *ad libitum*. Animals were left to acclimatize to laboratory conditions before starting the study. The care and handling of the animals were approved by the Animal Care and Use Committee at the Suez Canal University and were in accordance with Guide to the Care and Use of Experimental Animals (14).
All drugs and reagents were purchased from Sigma chemical co. Egypt.

Induction of type–2 DM in rats:

Fructose was administered to rats (66%, w/v solution, 5 ml/kg/day, p.o., for 8 w) to induce diabetes mellitus type-2 [15]. The animals with fasting blood glucose level more than 280 mg/dl were selected to be included in the study.

Experimental design:

The diabetic animals were arranged randomly to five groups (*n*=6).

Group I (normal control) - vehicle (distilled water; 5 ml/kg, p.o.),
Group II (diabetic control) - fructose (66% w/v solution, 5 ml/kg/day, p.o., for 12 w),
Group III -metformin+fructose (70 mg/kg, p.o+66% w/v p.o., for 12 w)
Group IV -, sitagliptin+fructose (20 mg/kg, p.o+66% w/v p.o., for 12 w),

Group V: sitagliptin+metformin+fructose (20 mg/kg, 70 mg/kg, p.o+66% w/v p.o.), for 12 w

Doses of drugs:

Sitagliptin 20 mg/kg [16], Metformin 70mg/kg [17]

All treatments were administered after induction of diabetes. Body weight of each animal was measured before the start of treatments and thereafter every four weeks of drug treatments. The systolic blood pressure by tail-cuff method. Blood samples were collected from retro-orbital plexus under ether anesthesia every four weeks for determination of serum glucose and serum creatinine.

Estimation of serum glucose, glycated hemoglobin:

The rats were anaesthetized under light ether; blood was removed from the retro orbital plexus using a capillary in micro sample tubes, serum was separated and used for biochemical investigations. Serum glucose, using standard biochemical kits.

Glycosated hemoglobin using HPLC method was measured at the beginning of the study and after 12 weeks [18]

Assessment of urine parameters

The urinary creatinine and creatinine in plasma were measured using an AU5800 automatic analyzer (Beckman Coulter, Inc., CA, USA). Creatinine clearance (CCr) and Urine albumin (mg/dL) = UACR in mg/g ~~ Albumin excretion in mg/day Urine creatinine (g/dL) UACR is a ratio between two measured substances. Unlike a dipstick test for albumin, it is unaffected by variation in urine concentration.; and CCr=urinary creatinine (UCr) (mg/ml) × urine volume (ml/kg)/creatinine in plasma (mg/ml) [19].

Determination of MDA and GSH-Px levels

One small section (200 mg) of left kidney was removed from the rats and weighed. Subsequently, saline was added according to the tissue weight: Saline volume=1:9 (w/v). then homogenization was done at 4°C by a DY89-I electric homogenate (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China), the homogenates were then centrifuged at 1,100xg for 15 min at room temperature., MDA content and GSH-Px (glutathione peroxidase) levels were determined by using commercially available kits, according to manufacturer's protocol.[20-21]

Histopathology and immunohistological examination

Kidney samples were fixed in 10% formalin, paraffin-embedded, cut into 5-micron sections and stained with hematoxylin and eosin and PAS stain (Sanpu Chemical Reagent Co., Ltd.). Slides were examined under light microscope (magnification, ×400; Nikon, Tokyo, Japan). All pathological procedures were done by a pathologist who is blind for the study groups.

Glomerular lesions was graded on scale based on Gellman criteria:

D0, all glomeruli appear normal; D1,some focal lesions appear on glomeruli; D2, mesangial thickening is present throughout the kidney diffusely; D3, lumen of capillary is narrowed and obliterated; D4, glomeruli are hylanized [20]. Tubulointerstitial damage was graded as follows: 0 – all tubules are normal; 1 – minimal injury is seen (single focus); 2 – mild injury (two isolated foci); 3 – moderate injury (five isolated foci); and 4 – severe injury (diffuse infiltration and fibrosis) [22]. Vascular lesions were graded as follows: 0 – normal blood vessels; 1–somefocal thickening of the walls of the capillaries; 2 – diffuse thickening of capillaries; 3 – obliterated lumen of some capillaries. Interstitial inflammation was graded as follows: 0 – no cell infiltration; 1 – minimal amount cell infiltration; 2 – mild cell infiltration; and 3 – diffuse infiltration[23].

ISSN: 0975-8585

Immuno-histochemistry for expression of renal laminin

Laminin was detected by rabbit polyclonal antibody anti-rat laminin (Dako Company, Egypt) using the technique of the heat-induced antigen retrieval . Intensity of laminin stain was performed on scale based on Taneda scale: 0 – no staining; 1 – mesangial staining involving < 25% of the area examined; 2 – segmental mesangial staining from 25 to 50% of mesangial areas is present; 3 – mesangial staining from 50 to 75% of the areas; 4 – staining more than 75% of areas examined [23]

There is no conflict of interest or funding agency for this work.

Statistical analysis:

Results are expressed as mean±SEM, and the statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by Dunnett's test. Probability level less than 0.05 was considered statistically significant.

RESULTS

Metformin group and sitagliptin group showed a significant decreased fasting blood sugar, body weight and systolic blood pressure as compared to diabetic group level. Combination of both drugs caused a significant decrease of FBS level, body weight and systolic blood pressure compared to treated groups and diabetic group and there is non significant difference between Metformin + Sitagliptin group and normal control at the end of the study (P value<0.05) which indicate better control of diabetes(fig.1 ,2,3).

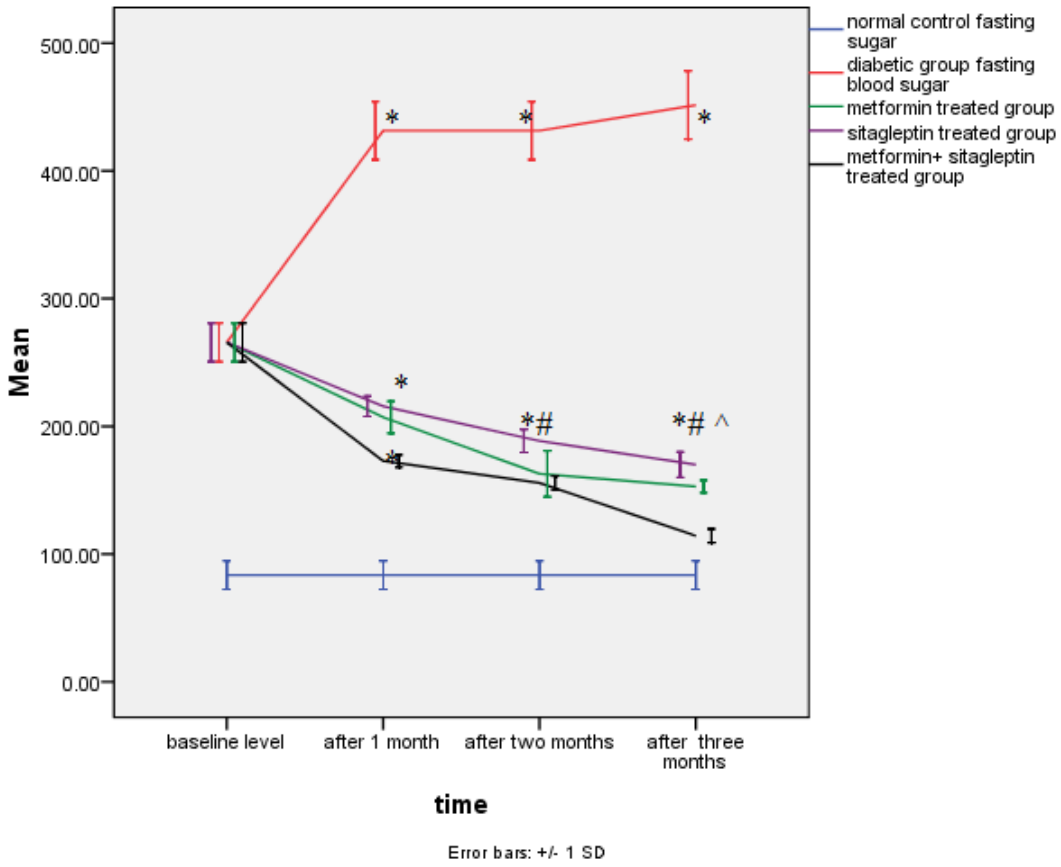

Fig 1: Time course monitoring of fasting blood sugar in study groups (Mean ±SD) P value<0.05 * Significant versus normal control, # versus diabetic control,^ versus metformin+sitaglitin group

ISSN: 0975-8585

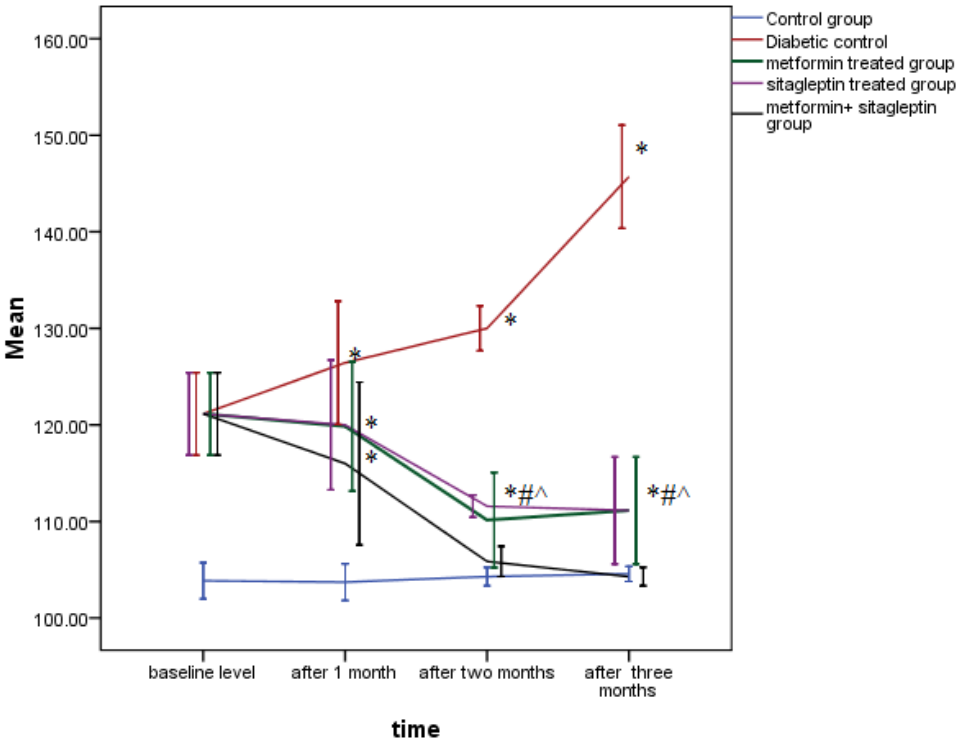

Fig 2: Time course monitoring of systolic blood pressure in study groups (Mean ±SD) P value<0.05* Significant versus normal control, # versus diabetic control,^ versus metformin+sitagliptin group

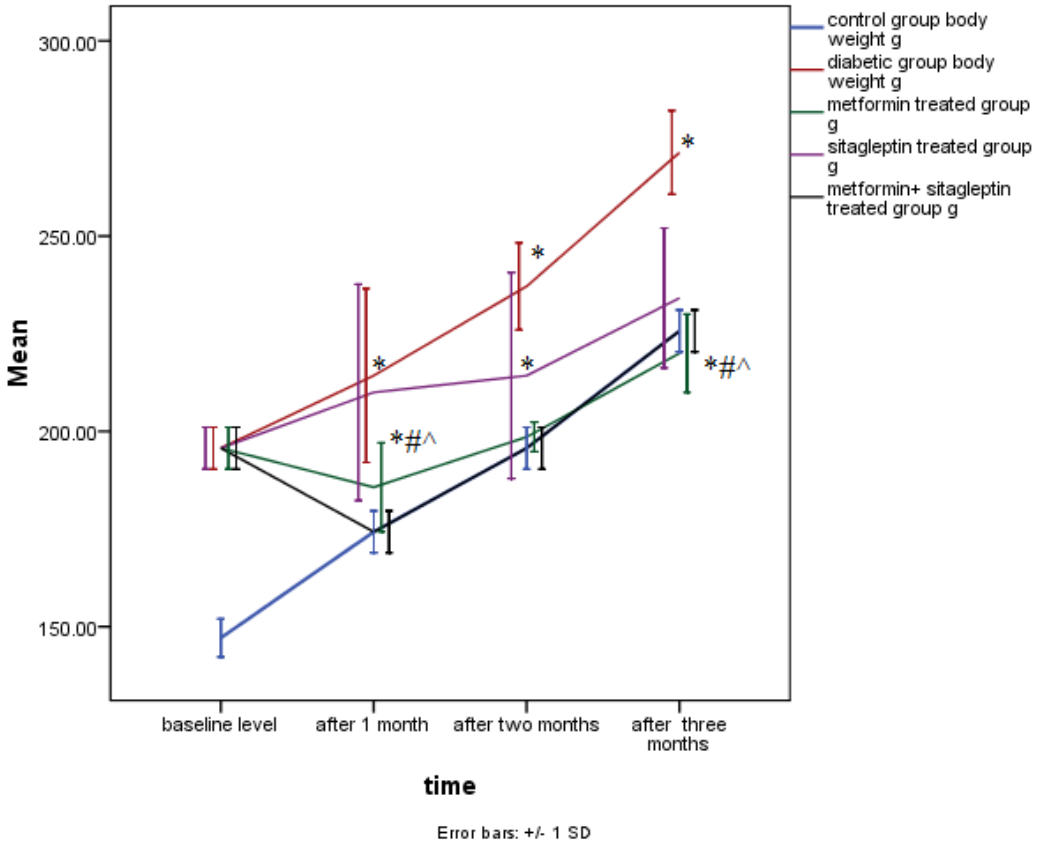

Fig 3: Time course monitoring of body weight in study groups (Mean ±SD) P value<0.05 * Significant versus normal control, # versus diabetic control,^ versus metformin+sitagliptin group

ISSN: 0975-8585

The level of glycosated hemoglobin at the end of the study showed a significant decrease in metformin and sitagliptin groups compared to diabetic group and more decrease in combination group compared to treated groups (p value<0.05) (fig.4.)

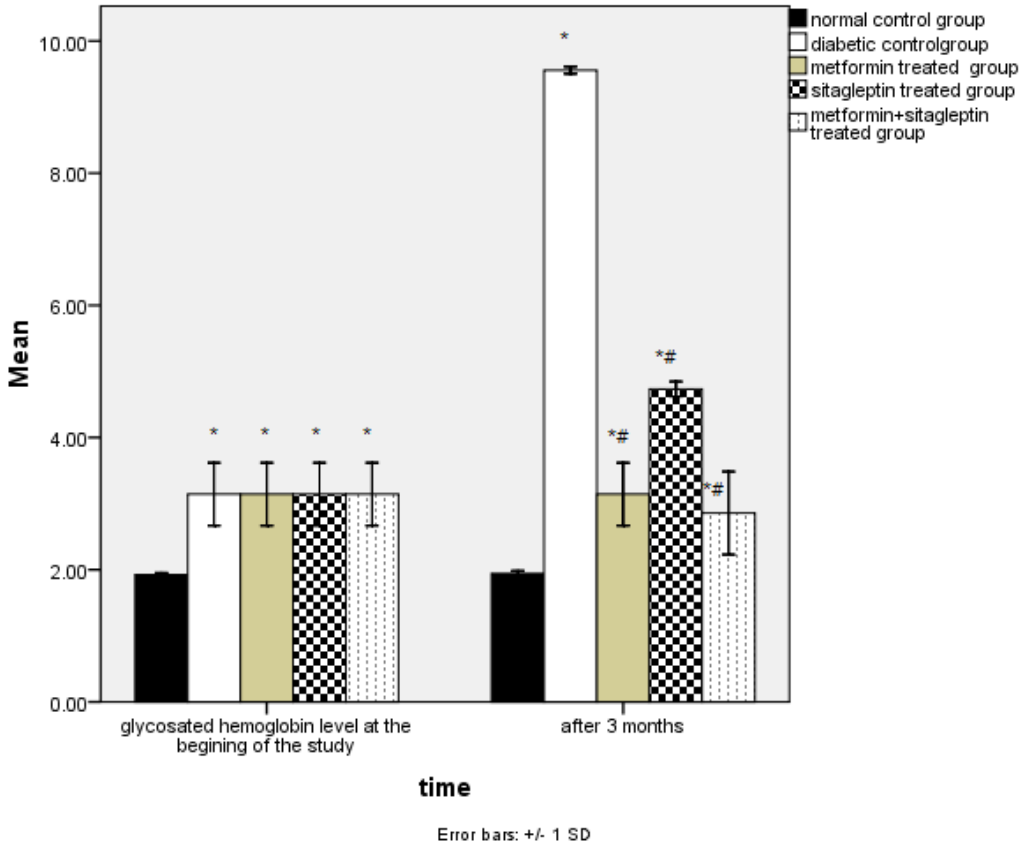

Fig. 4: level of glycosated hemoglobin in study groups (Mean ±SD)
*** Significant versus normal control, # versus diabetic control,^ versus metformin+sitagliptin group**

The level of UACR in treated groups chowed significant decrease compared to diabetic group but combination of both drugs caused more significant decrease in the level than the use of one drug alone , there was non significant difference between metformin+sitagliptin group and normal control group (p value ,0.05), (fig.5). creatinine clearance in all treated groups showed significant decrease compared to diabetic group and non significant difference between treated groups and normal control group (fig.6).

ISSN: 0975-8585

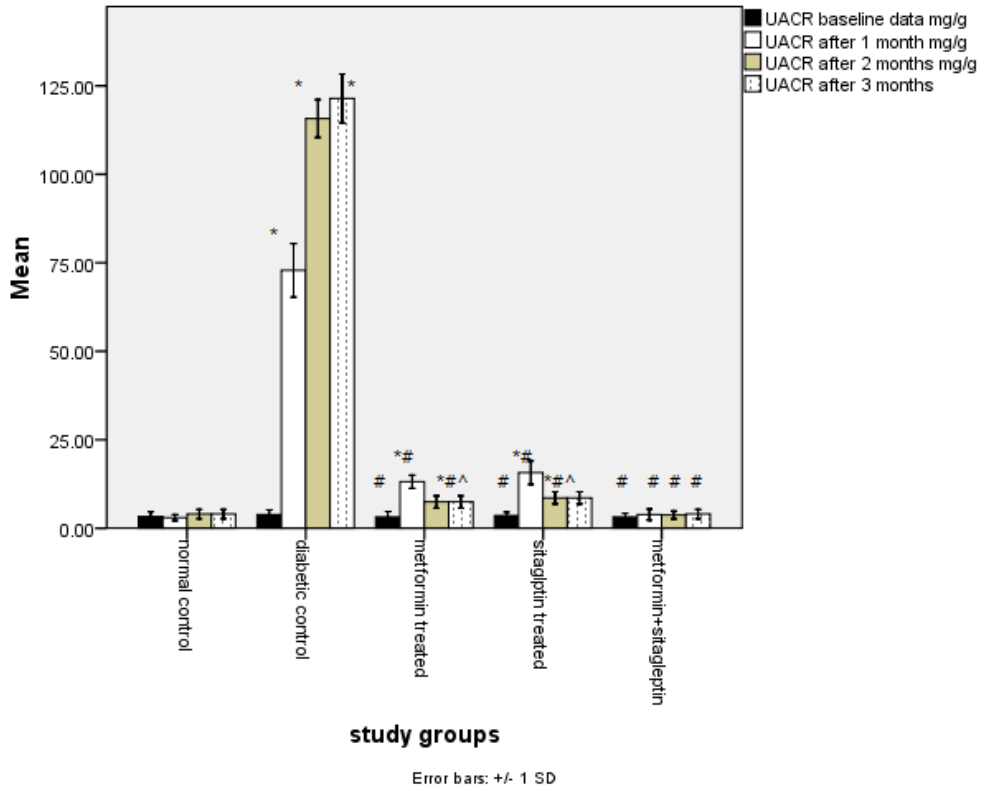

Fig 5: Level of UACR in study groups at different time intervals (Mean ±SD) P value<0.05 * Significant versus normal control, # versus diabetic control,^ versus metformin+sitagliptin group

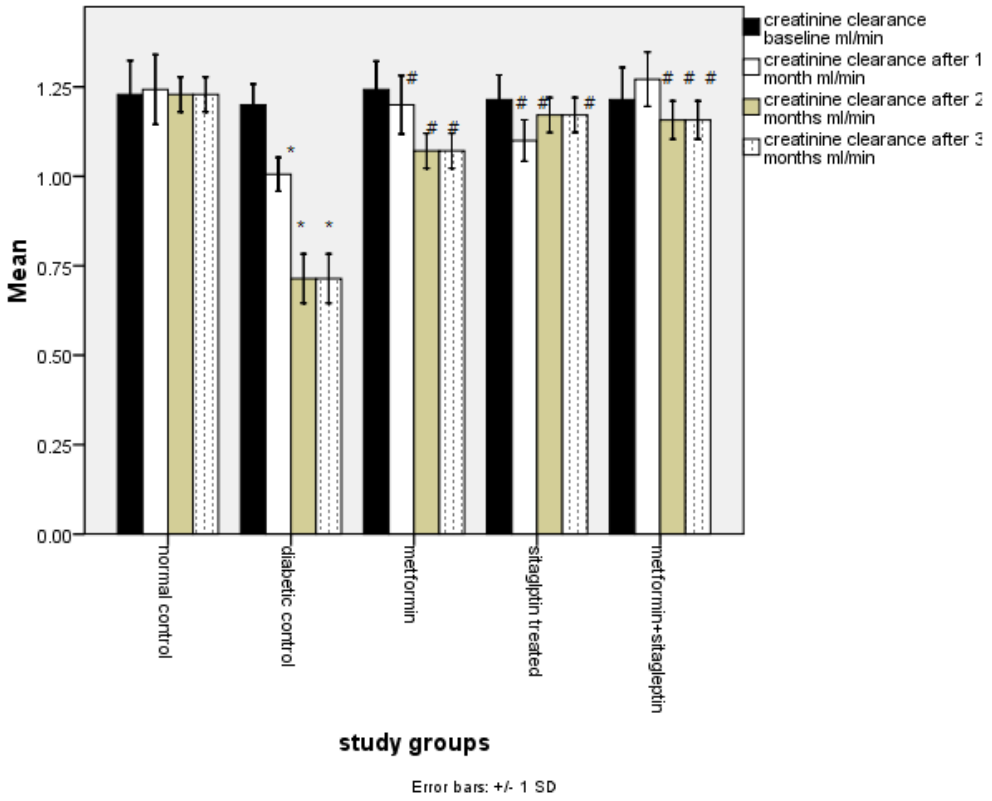

Fig 6: creatinine clearance in study groups at different time intervals (Mean ±SD) P value<0.05 * Significant versus normal control, # versus diabetic control,^ versus metformin+sitagliptin group

ISSN: 0975-8585

There was a significant increase in diabetic control, metformin treated and sitagliptin treated groups in LDL,TG and TC level and decrease in HDL level compared to normal control and combination group, although there was a significant difference in treated groups compared to diabetic group but combination of both drugs caused normalizations of all values p value< 0.05. (table 1).

Table 1: level of blood lipids in study groups at the end of the study

Study groups	LDL	HDL	TG	TC
Normal control	0.55±0.07	0.67±0.07	0.28±0.04	1.59±0.10
Diabetic control	1.22±0.09*	0.40±0.04*	0.48±0.04*	2.30±0.1*
Metformin treated	0.75±011*^#	0.50±0.05*^#	0.40±0.03*^#	1.80±0.13*^#
Sitagliptin treated	0.70±012*^#	0.45±0.04*^#	0.41±0.02*^#	1.75±0.12*^#
Metformin+sitagliptin	0.60±013*	0.62±0.05*	0.27±0.02*	1.60±0.11*

(Mean ±SD) P value<0.05 * Significant versus normal control, # versus diabetic control,^ versus metformin+sitagleptin group

There was a significant decrease in antioxidant activity as shown by decreased glutathione perioxidae GSH-Px and increased MDA in the kidney of diabetic rats compared to normal and treated groups. There was non significant difference in GSH-Px and MDA level in treated groups compared to normal (p value <0.05) indicating improvement of antioxidant activity in these groups(fig.7).

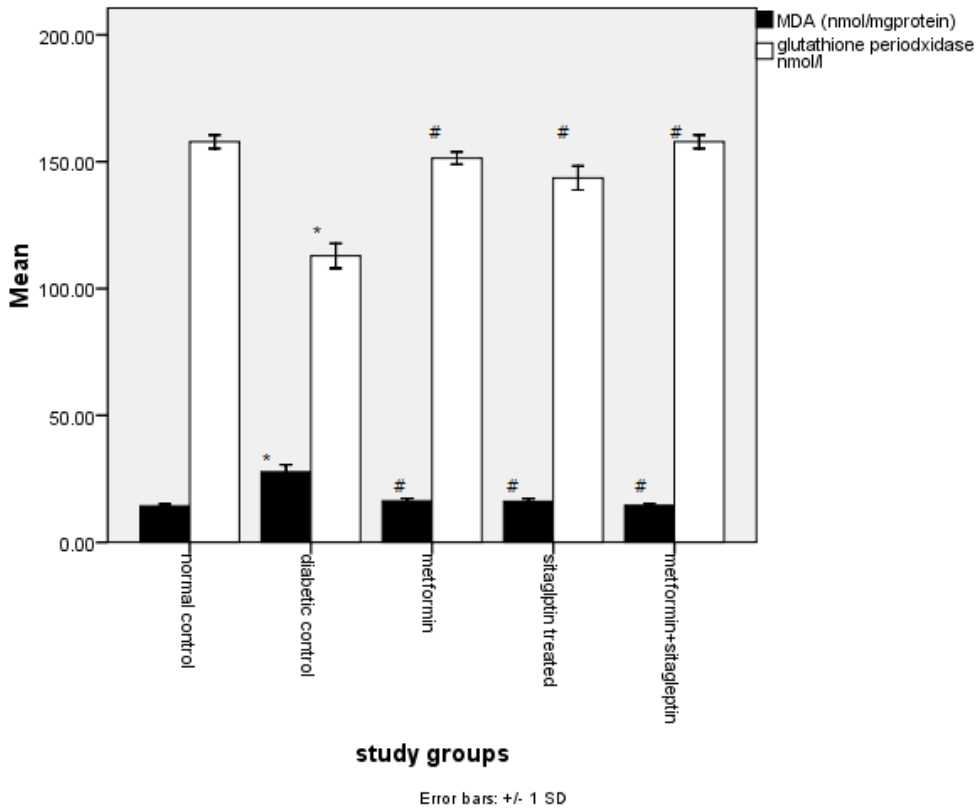

Fig 7: level of GSH-Px and MDA in the kidney of study groups(Mean ±SD) P value<0.05 * Significant versus normal control, # versus diabetic control,^ versus metformin+sitagliptin group

Pathological changes appeared in diabetic kidney manifested by glomerular lesions in the form of hypertrophied thichened glomeruli with thich basement membranes and hylanosis of some glomeruis.

ISSN: 0975-8585

There was interstitial inflammation and interstitial lesions and IFTA (interstitsial fibrosis and tubular atrophy) in diabetic control group, There was also vascular lesions grade II (hylanosis of afferent and efferent arterioles) .Diabetic group lesions were statistically significant (p value < 0.05) versus normal control group.

Either Metformin or Sitagliptin treated group showed normal glomeruli but showed focal glomerular lesions and interstitial inflammation with focal vascular lesions or and minimal IFTA there was significant difference between these groups and normal control group but there was also significant decrease in pathologic changes versus diabetic group

There was significant decrease in pathological changes in combination group versus treated groups and non significant difference versus normal indicating that combination between metformin and sitagliptin caused better prevention of diabetic changes (fig. 8-9-10).

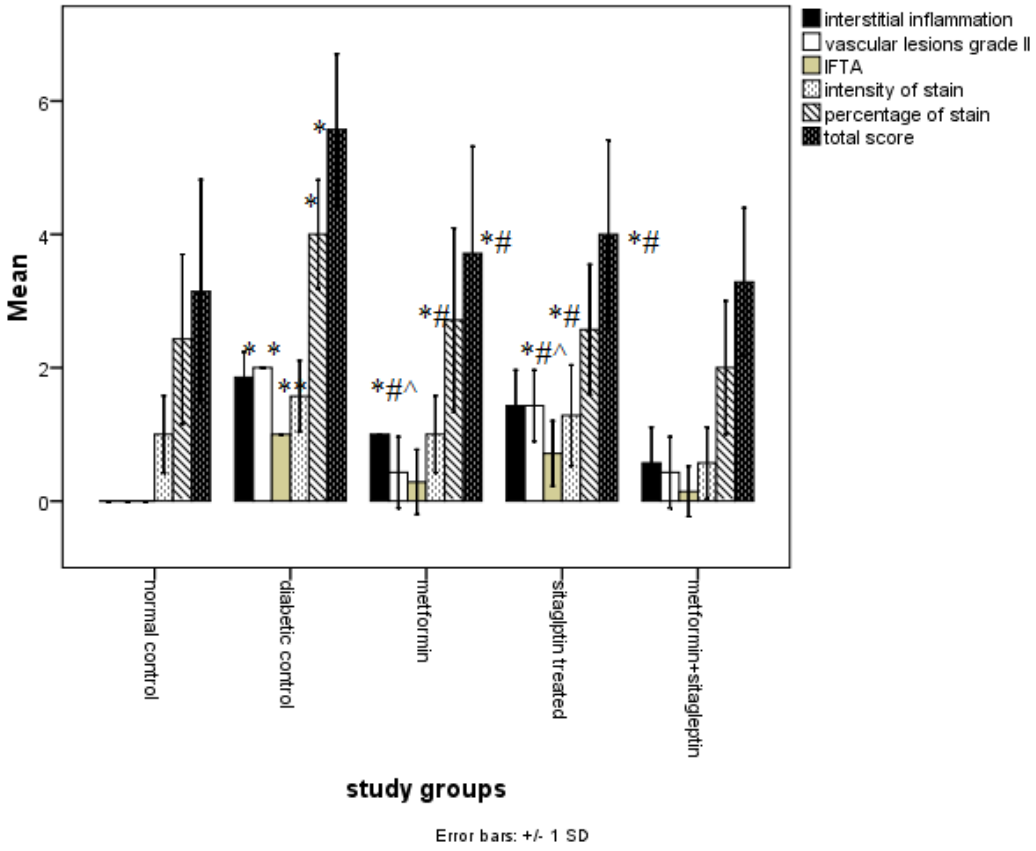

Fig 8: Score of pathological changes in study groups
(Mean ±SD) P value<0.05 * Significant versus normal control, # versus diabetic control,^ versus metformin+sitagliptin group

ISSN: 0975-8585

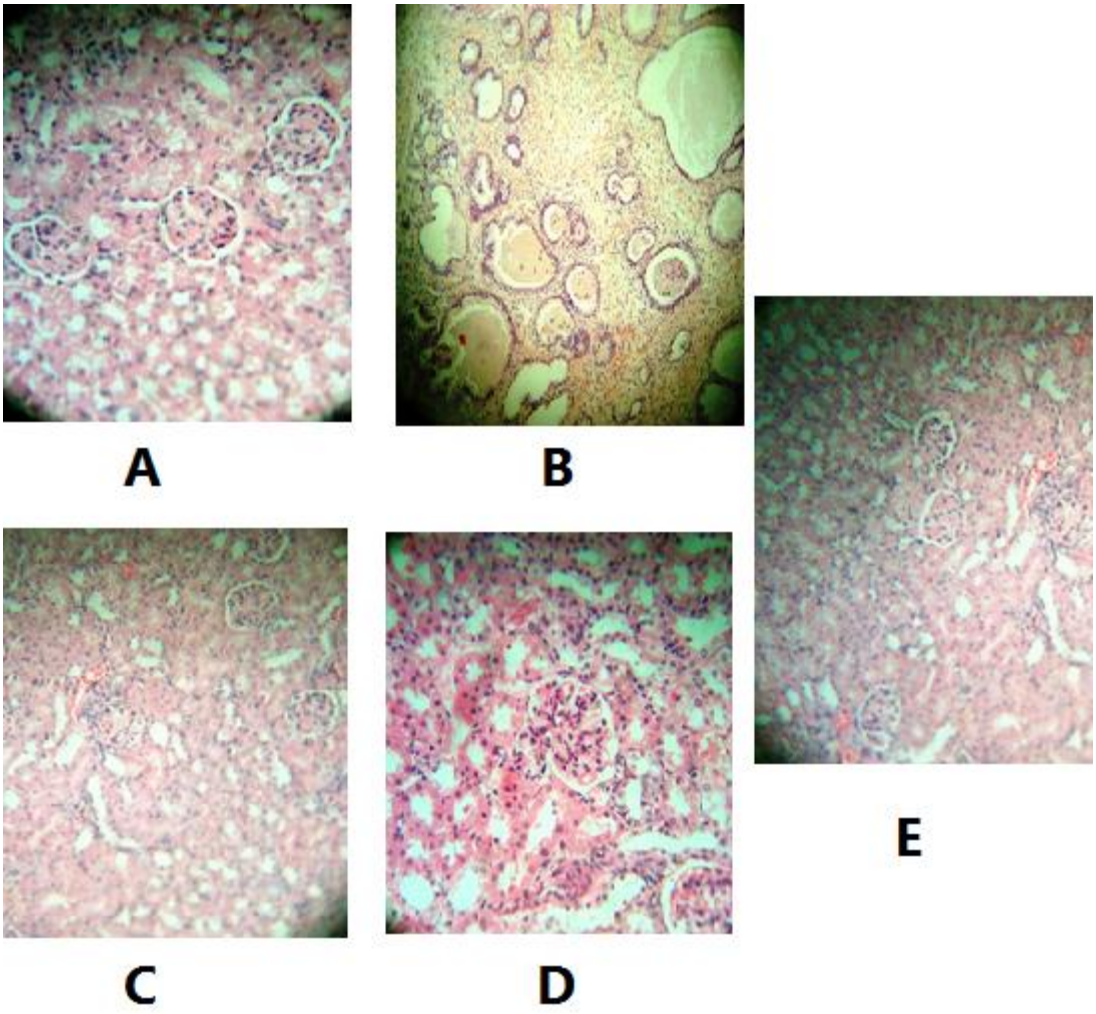

Fig 9: Pathological changes in study groups H and E stain×40
A: normal control B: diabetic group C: metformin treated group
D Sitagliptin treated group E: metformin+sitagliptin group

ISSN: 0975-8585

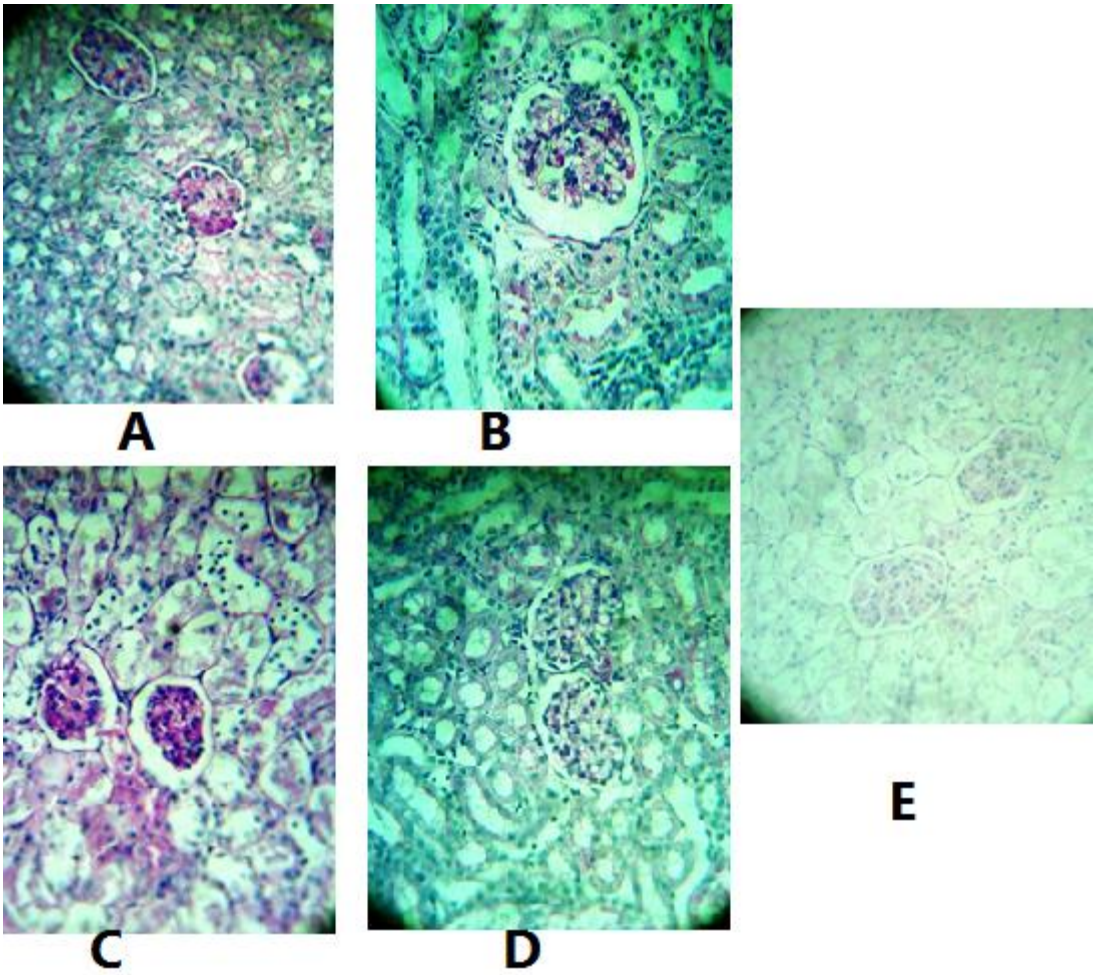

Fig 10: the histological morphology of kidneys stained by PAS stain
A: normal control B: diabetic group C: metformin treated group D Sitagliptin treated group
E: metformin+sitagliptin group

Table 2: immunohistological stain of laminin in study groups:

study groups	normal control	Diabetic control	Metformin treated	Sitagliptin treated	Metformin+ sitagliptin
Intensity of stain	1±.37	3.15±.37*	1.3±.57#	1.28±.48#	1.14±.37#

A: normal control B: diabetic group C: metformin treated group D Sitagliptin treated group E :metformin+sitagliptin group

Intensity of immunostain of laminin stain showed significant increased stain in diabetic group compared to normal but treated groups showed non significant change in intensity of stain versus normal (p value <0.05) indicating thickening of basement membrane and accumulation of extracellular matrix whereas treated groups showed normal intensity of stain (table 2, fig.11) .

ISSN: 0975-8585

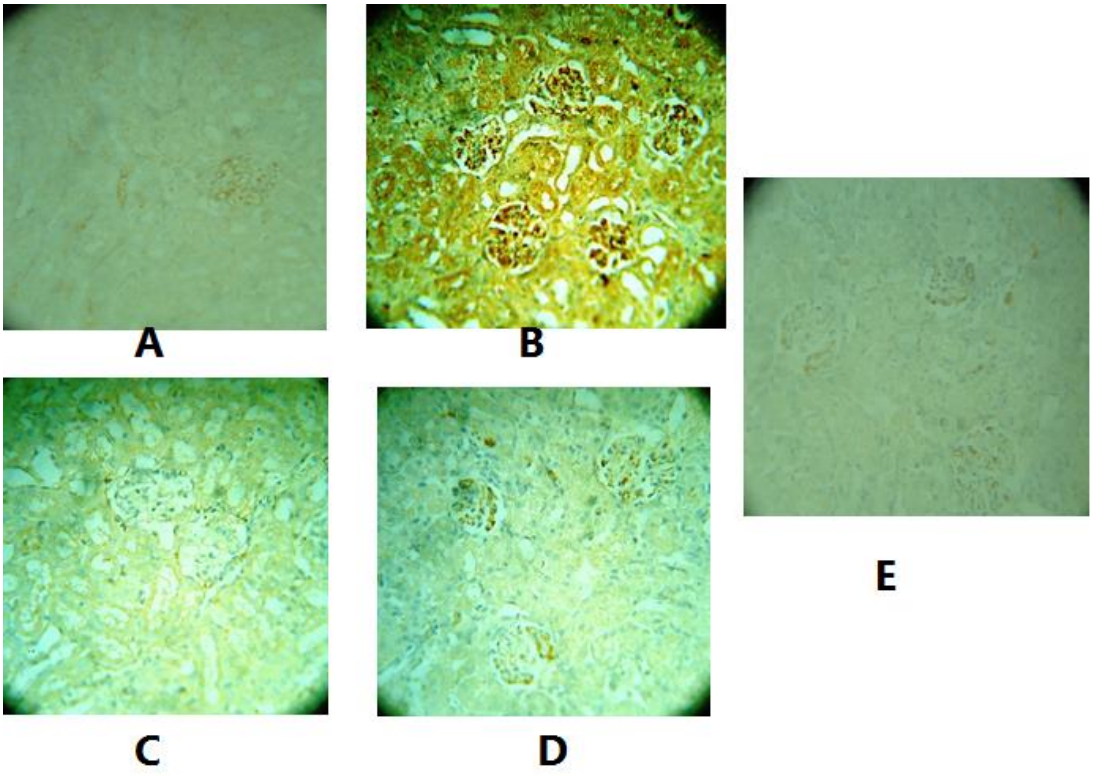

Fig 11: Intensity of lamnin stain in study groups.

A: normal group B: diabetic control C: metformin treated D:Sitagliptin treated E: metformin+sitagliptin treated group

DISCUSSION

In this study, we studied the effect of metformin and sitagliptin on oxidative stress in diabetic nephropathy in rats with type 2 diabetes, diabetes was induced by high fructose adminostration. Insulin resistance, hyperinsulinemia, and hypertriglyceridemia occur in a short time when normal rats are administered a high fructose diet, as has been seen previous studies [15][24][25].

The results of our study showed that merformin and sitagliptin markedly improved the renal lesions and ameliorate the GBM thickness of the kidney in diabetic rats. At the same time, they can decrease the FBG levels, reduce blood pressure, decrease body weight, increase creatinine clearance and decreased UACR levels, decrease MDA level in renal tissue and increase glutathione peroxidase level. Combination of the two drugs caused significant effect on the parameters measured more than each drug alone. Combined treatment with metformin or sitagliptin caused glycemic control, anti-oxidative and anti-inflammatory effects in the treatment of rats with induced type 2 diabetes.

There was also significant increase in lipid profile in diabetic control group compared to normal and treated groups and decrease in HDL, but combination of both drugs caused significant improvement of lipid profile compared to each drug alone.

Increased plasma glucose levels successfully caused renal injury that was like that present in patients with diabetic nephropathy, hyperglycemia causes oxidative stress and increases renal injury [26].

Renal injury was assessed through kidney function assessments, including elevated declined CCr levels and presence of albumin in the urine is which is considered a primary marker of kidney damage in the early diagnosis of DN, and the urinary albumin is usually measured to assess renal lesions in diabetic individuals [27-28]. Creatinine clearance rate from blood to urine (CCr) depends on glomerular filtration rate [29]; therefore, CCr is usually used as a parameter for assessment of kidney function. Kidney damage

parameters were significantly increased in the diabetic group, as compared with the control group. Treatment with metformin or Sitagliptin either alone or in combination decreased kidney dysfunctions, indicating the protective effects of these drugs administration in rats with type2 diabetes. In the diabetic group, morphological and ultrastructural analysis showed severe damage to renal tissue, manifested by distorted glomeruli, dilation of the renal capsule and fibrosed kidney tubules, GBM thickening and extracellular matrix accumulation manifested by increased lamninimmunostain. Treated groups showed mild morphological alterations.

Elevated glucose level is the main pathologic factor of DM which causes diabetic complications. Chronic elevated blood sugar causes accumulation of advanced glycation end products (AGEs) in body tissues, which contribute to vascular complications in diabetic patients [30]. A previous study proved that AGEs are usually present in diabetic patients with kidney dysfunction, leading to the overproduction of free radicles and oxidants[31]. Free radicals and oxidants production as a result of hyperglycemia are responsible for damaging cell membranes and inactivation of endogenous antioxidants, lipid and carbohydrate [32-33], and they are the main cause responsible fordiabetic-related complications [34-35]. Endogenous antioxidant molecules, such as GSH-Px, counteract free radicles-mediated renal injury [36]; however, they are largerly decreased in patients with Type 2 diabetes, indicating oxidative stress [37-38]. Therefore, strict glucose control is the most important aim of the therapy, as it leads to decrease oxidative stress [39-40]. In this study, elevated FBG levels were significantly reduced by metformin and Sitagliptin. The results indicated that glycemic control is important for the renoprotective effect. Furthermore, the levels of GSH-Px and MDA in kidney homogenates were determined. In the diabetic group, decreased level of the GSH-Px antioxidants were accompanied by an increase in MDA levels. Combined treatment with metformin or Sitagliptin increased GSH-Px levels and reduced MDA levels.

These results concluded that combined treatment with metformin and sitagliptin exerts more renoprotective effect by increasing GSH-Pxand reducing MDA more than treatment than each drug alone, thus combination of metformin and sitagliptin is recommended for treatment of type 2 diabetes and prevention of diabetic nephropathy.

REFERENCES

[1] Zou J, Yu X, Qu S, Li X, Jin Y, Sui D. Protective effect of total flavonoids extracted from the leaves of Murrayapaniculata (L.) Jack on diabetic nephropathy in rats. Food ChemToxicol. 2014;64:231–237.

[2] Gohda T, Mima A, Moon JY, Kanasaki K. Combat diabetic nephropathy: From pathogenesis to treatment. J Diabetes Res. 2014;2014:207140.

[3] Pan HZ, Zhang L, Guo MY, Sui H, Li H, Wu WH, Qu NQ, Liang MH, Chang D. The oxidative stress status in diabetes mellitus and diabetic nephropathy. ActaDiabetol. 2010;47(Suppl 1):S71–S76.

[4] Swaminathan S, Shah SV. Novel approaches targeted toward oxidative stress for the treatment of chronic kidney disease. CurrOpinNephrolHypertens. 2008;17:143–148.

[5] Tabak O, Gelisgen R, Erman H, Erdenen F, Muderrisoglu C, Aral H, Uzun H. Oxidative lipid, protein and DNA damage as oxidative stress markers in vascular complications of diabetes mellitus. Clin Invest Med. 2011;34:E163–E171.

[6] Ho C, Lee PH, Hsu YC, Wang FS, Huang YT, Lin CL. Sustained Wnt/β-catenin signaling rescues high glucose induction of transforming growth factor-β1-mediated renal fibrosis. Am J Med Sci. 2012;344:374–382.

[7] Zorena K, Malinowska E, Raczyńska D, Myśliwiec M, Raczyńska K. Serum concentrations of transforming growth factor-Beta 1 in predicting the occurrence of diabetic retinopathy in juvenile patients with type 1 diabetes mellitus. J Diabetes Res. 2013;2013:614908.

[8] Chen MM, Lam A, Abraham JA, Schreiner GF, Joly AH. CTGF expression is induced by TGF-beta in cardiac fibroblasts and cardiac myocytes: A potential role in heart fibrosis. J Mol Cell Cardiol. 2000;32:1805–1819.

[9] Li X, Cui X, Sun X, Li X, Zhu Q, Li W. Mangiferin prevents diabetic nephropathy progression in streptozotocin-induced diabetic rats. Phytother Res. 2010;24:893–899.

[10] Nasri H, Rafieian-Kopaei M. Metformin: Current knowledge. J Res Med Sci. 2014;19:658–664.

[11] Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: An update. Ann Intern Med. 2002;137:25–33.

[12] Ballav C, Gough SC. Safety and efficacy of sitagliptin-metformin in fixed combination for the treatment of type 2 diabetes mellitus. Clin Med Insights Endocrinol Diabetes. 2013;6:25–37.

[13] Nade VS, Kawale LA, Patel KM. Protective effect of sitagliptin and rosuvastatin combination on vascular endothelial dysfunction in type-2 diabetes. Indian J Pharm Sci. 2015;77:96–102.
[14] Guide to the Care and Use of Experimental Animals . 1993; Vol. 1, 2nd ed..
[15] Hwang IS, Ho H, Hoffman BB, Reaven GM. Fructose-induced insulin resistance and hypertension in rats. Hypertension. 1987;10:512–6
[16] Vandana S. Nade, L. A. Kawale, and K. M. Patel.Protective Effect of Sitagliptin and Rosuvastatin Combination on Vascular Endothelial Dysfunction in Type-2 Diabetes. Indian J Pharm Sci. 2015 Jan-Feb; 77(1): 96–102.
[17] Siwei Zhang, HualiXu, Xiaofeng Yu, Yi Wu, and Dayun Sui.Metformin ameliorates diabetic nephropathy in a rat model of low-dose streptozotocin-induced diabetes. ExpTher Med. 2017 Jul; 14(1): 383–390
[18] Detata V, Novelli M, Bombara M: Determination of glycatedhemoglobins in the rat: comparison between two different chromatographic methods and application in experimental diabetology. Res Exp Med (Berl), 1996, 196, 9–16.
[19] Kong LL, Wu H, Cui WP, Zhou WH, Luo P, Sun J, Yuan H, Miao LN. Advances in murine models of diabetic nephropathy. J Diabetes Res. 2013;2013:797548. –
[20] Janero DR. Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injure. Free Rad Bio Med 1990; 9: 515-40.
[21] Pascual P, Martinez-Lara E, Bárcena JA, López-BareaJ,Toribio F. Direct assay of glutathione peroxidase activity using high-performance capillary electrophoresis. J Chromatogr 1992; 581: 49-56.
[22] Gellman D, Pirani C, Soothill G, Muethrcke R, Kark R: Diabetic nephropathy: clinical and pathologic studies based on renal biopsy. Med J, 1959, 38, 312–317.
[23] Taneda S, Jeffery W, Pippin B: Amelioration of diabetic nephropathy in SPARC-null mice. J Am SocNephrol, 2003, 14, 968–980.
[24] Zavaroni I, Sanders S, Scott S, Reaven GM. Effect of fructose feeding on insulin secretion and insulin action in the rat. Metabolism 1980;29:970-973
[25] Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50:537–546.
[26] Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51:216–226.
[27] Hall-Craggs M, Brenner DE, Vigorito RD, Sutherland JC. Acute renal failure and renal tubular squamous metaplasia following treatment with streptozotocin. Hum Pathol. 1982;13:597–601
[28] Tay YC, Wang Y, Kairaitis L, Rangan GK, Zhang C, Harris DC. Can murine diabetic nephropathy be separated from superimposed acute renal failure? Kidney Int. 2005;68:391–398.
[29] Jiang X, Ma H, Wang Y, Liu Y. Early life factors and type 2 diabetes mellitus. J Diabetes Res. 2013;2013:485082.
[30] Lee KM, Yang SJ, Kim YD, Choi YD, Nam JH, Choi CS, Choi HS, Park CS. Disruption of the cereblon gene enhances hepatic AMPK activity and prevents high-fat diet-induced obesity and insulin resistance in mice. Diabetes. 2013;62:1855–1864.
[31] Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. Diabetes. 1983;32(Suppl 2):S64–S78. doi: 10.2337/diab.32.2.S6
[32] Viberti G, Wheeldon NM, MicroAlbuminuria Reduction With VALsartan (MARVAL) Study Investigators Microalbuminuria reduction with valsartan in patients with type 2 diabetes mellitus: A blood pressure-independent effect. Circulation. 2002;106:672–678.
[33] Wang XL, Lu JM, Pan CY, Tian H. A study comparing the prevalence of urinary albumin excretion and microalbuminuria in pre-diabetes subjects. ZhonghuaNeiKeZaZhi. 2004;43:170–173.
[34] Stirban A, Gawlowski T, Roden M. Vascular effects of advanced glycationendproducts: Clinical effects and molecular mechanisms. MolMetab. 2013;3:94–108.
[35] Sakai H, Jinde K, Suzuki D, Yagame M, Nomoto Y. Localization of glycated proteins in the glomeruli of patients with diabetic nephropathy. Nephrol Dial Transplant. 1996;11(Suppl 5):S66–S71. doi: 10.1093/ndt/11.supp5.66.
[36] Rösen P, Nawroth PP, King G, Möller W, Tritschler HJ, Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications: A summary of a Congress Series sponsored by UNESCO-MCBN, the American diabetes association and the german diabetes society. Diabetes Metab Res Rev. 2001;17:189–212. doi: 10.1002/dmrr.196.
[37] Iwasaki Y, Sawada T, Kijima H, Kosuge T, Katoh M, Rokkaku K, Kita J, Shimoda M, Kubota K. Estimated glomerular filtration rate is superior to measured creatinine clearance for predicting postoperative renal dysfunction in patients undergoing pancreas to duodenectomy. Pancreas. 2010;39:20–25.

[38] Yan LJ. Analysis of oxidative modification of proteins. CurrProtoc Protein Sci Chapter. 2009;14:Unit14.4.
[39] Booth AA, Khalifah RG, Todd P, Hudson BG. In vitro kinetic studies of formation of antigenic advanced glycation end products (AGEs). Novel inhibition of post-Amadoriglycation pathways. J Biol Chem. 1997;272:5430–5437.
[40] Miyoshi H, Taguchi T, Sugiura M, Takeuchi M, Yanagisawa K, Watanabe Y, Miwa I, Makita Z, Koike T. Aminoguanidinepyridoxal adduct is superior to aminoguanidine for preventing diabetic nephropathy in mice. HormMetab Res. 2002;34:371–377.