ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Cardio, Neuro And Renoprotective Activities of Atorvastatin in Streptozotocin-Induced Type2 Diabetic Rats Undergoing Treatment with Metformin and Glimepiride.

Peddolla R, Rondi S and Venisetty RK*

Department of Pharmacology, St. Peter's Institute of Pharmaceutical Sciences, Warangal, Andhra Pradesh, India.
*Chaitanya College of Pharmacy Education and Research, Hanamkonda, Warangal 506001, Andhra Pradesh, India.

ABSTRACT

Diabetes was induced by streptozotocin (50 mg/kg, i.p.) in the adult Wistar Albino rats. Diabetic rats were treated according to their grouping and the treatment protocol i.e., (i) atorvastatin (10 mg/kg/d), (ii) metformin (120 mg/kg/d) + glimepiride (1 mg/kg/d), (iii) atorvastatin (10 mg/kg/d) + metformin (120 mg/kg/d) + glimepiride (1 mg/kg/d) for 6 weeks. After the treatment, nerve fiber conduction was assessed by a thermal stimulus model (tail-withdrawal test). Serum levels of urea, creatinine and total protein were estimated as renal function markers. Serum lipids (LDL, triglycerides and HDL) and CK-MB were estimated to ascertain cardioprotective activity. Antioxidant enzymes (glutathione peroxidase and catalase) and the oxidative stress marker (MDA) were estimated in the kidney and the brain homogenates of diabetic rats. Results obtained, inferred that the combination of atorvastatin with oral hypoglycemic agents (triple therapy) improved glycemic control and the antioxidant defenses. Enhanced nerve fiber functioning, substantial decrease in cardiac risk markers (LDL, triglycerides and CK-MB) and reduced protein overload, in comparison to glimepiride plus metformin therapy in streptozotocin-induced type 2 diabetic Wistar Albino rats was observed.

Keywords: Antioxidant defense, Atorvastatin, Cardioprotection, Diabetes, Neuroprotection, Pleiotropic effects, Protein overload, Renoprotection.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Type 2 diabetes is a chronic progressive disease which leads to a variety of debilitating complications and vital organ damage. Regular monitoring of blood glucose levels alone is inadequate in the management of diabetes and its complications like cardiomyopathy, retinopathy, nephropathy and neuropathy [1].

Diabetes is the prominent risk factor for coronary heart disease (CHD). In diabetic patients, CHD associated mortality is 2 to 4 times higher when compared with nondiabetics [2]. Acceptable glycemic control is needed but provides limited benefit against CHD prevention. In case of type 2 diabetic patients, significant lipid lowering is pivotal to curb dyslipidemia [3]. In general, statin should be a first line of agent to treat diabetic dyslipidemia [2, 4].

Micro and macrovascular endothelial dysfunction and dyslipidemia accompanied by diabetes appear to affect nerve fiber function. Oxdative stress-related cellular damage and glycation of lipoproteins in diabetes precipitate endothelial dysfunction which in turn leads to atherosclerotic damage of the vasculature [5]. Treatment with lipid-lowering drugs would be expected to prevent this oxidative cellular damage and the consequent vascular complications [6].

In type 2 diabetic patients, renal dysfunction is ascribed mainly to proteinuria. Over activity of polyol pathway, glycation of essential endogenous macromolecules and high blood glucose levels play a prominent role in the pathogenesis of diabetic nephropathy [7]. All the risk factors and the potential mechanisms underlying the pathogenesis of diabetic complications are entangled with each other and hence targeting pivotal pathogenic mechanisms prevents the progression of diabetic complications, thereby prevents the vital organ damage.

In regular clinical practice, type 2 diabetic patients are treated with a combination of oral hypoglycemic agents. Progressive nature of the disease and inefficient glycemic control over time are the main reasons for this combination therapy [8]. This combination therapy of oral hypoglycemic agents is no longer able to prevent the disease progression with mere glycemic control. The addition of statins with different mode of action and with reported, beneficial pleiotropic effects may lead to desirable metabolic control and thereby prevents the vital organ damage [6].

Metformin acts by increasing the sensitivity of insulin and it is able to reduce the concentration of free fatty acids by 10 to 30%. In case of type 2 diabetic patients with obesity, metformin is the drug of choice [9].

Glimepride is an insulin secretogogue which provides glycemic control predominantly via extra pancreatic effects and hence it is devoid of unwanted hypoglycemia [10].

Statins possess multiple phenotypic, beneficial pleiotropic effects in addition to their lipid lowering property such as anti-inflammatory action, property to reverse endothelial

dysfunction, antioxidant action, ability to provide plaque stability, antiplatelet activity, etc., which make statins more suitable agents to be combined with oral hypoglycemic agents to provide protection against devastating diabetic complications [11].

MATERIALS AND METHODS

Animals

Wistar Albino rats weighing 160-180 g were used for the present study, procured from Sanzyme Ltd, Hyderabad, India. The animals were housed in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than six animals per cage, at an ambient temperature of 25± 2^0C with 12-h-light/12-h-dark cycle. Rats were given pellet diet and water *ad libitum*. The maintenance and the handling of animals were performed according to the rules and regulations of Institutional animal Ethical Committee, (Protocol No: 14/SPIPS/IAEC/12) Kakatiya University.

Drugs and solutions

Atorvastatin and metformin gift samples are obtained from Dr. Reddy's Laboratories Limited, Hyderabad, India, glimepiride from Aurobindo Pharma Limited, Hyderabad, India and streptozotocin was purchased from Himedia Research Laboratories, Mumbai, India. All the drugs except streptozotocin, were dissolved separately in 1% solution of sodium carboxymethyl cellulose (Sodium CMC) and were administered per orally (p.o.) to the diabetic animals according to their treatment protocol. Streptozotocin was dissolved in freshly prepared, ice cold, 0.1M citrate buffer pH (4.5) immediately before administration to the rats for the induction of diabetes.

Experimental induction of type 2 diabetes

In the experimental animals, diabetes was induced by the administration of streptozotocin at a dose of 50 mg/kg through intraperitoneal (i.p.) route [12]. Blood samples were collected from the retro orbital sinus 72 h after the administration of streptozotocin and the glucose levels were measured. Only the animals showing blood glucose levels more than 250 mg/dl were considered type 2 diabetic, and were taken into the study.

Experimental groups

Type 2 diabetic animals were divided into five groups (n=6), of which one group of nondiabetic healthy animals were taken as normal control, another group is the diabetic control and remaining three were the drug treatment groups.

Treatment schedule

Drug treatment (i) atorvastatin (10 mg/kg/d), (ii) metformin (120 mg/kg/d) + glimepiride (1 mg/kg/d), (iii) atorvastatin (10 mg/kg/d) + metformin (120 mg/kg/d) + glimepiride (1 mg/kg/d) was given daily in the morning to the diabetic animals for 6 weeks

using an oral gavage needle. Control animals were given purified water during this treatment period.

Parameters estimated

After the completion of drug treatment schedule, all the animals were subjected to tail immersion test using warm water (50 ^{o}C) and the tail withdrawal latency time was observed to assess the nerve fiber function.

Following day, blood samples were collected by means of retro orbital puncture from all groups of animals for the estimation of different biochemical parameters such as glucose, creatine kinase- MB, serum lipids (LDL, HDL, and triglycerides), creatinine, urea, and total protein using semi autoanalyser (Biochemical systems international) and the corresponding diagnostic kits (Crest biosystems, Tulip group).

The very next day, all groups of animals were sacrificed by cervical dislocation. Thereafter brain and kidney from each animal was harvested carefully, rinsed with ice- cold saline. Then the organs were homogenized with ice-cold phosphate buffer (pH 7.4). The resulting homogenates (10% w/v) were then centrifuged at 10,000 *g* for 15 min and the supernatant so obtained was used for the estimation of antioxidant parameters such as malondialdehyde (MDA), glutathione peroxidase (GPx), and catalase (CAT) with the help of UV- Visible spectrophotomer (ELICO limited).

Statistical analysis

Results were expressed as mean ± SEM. Analysis of the data was done by one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test using GraphPad Prism software version 5.0. A probability of $p<0.05$ was considered as statistically significant.

RESULTS

Effect on blood glucose levels

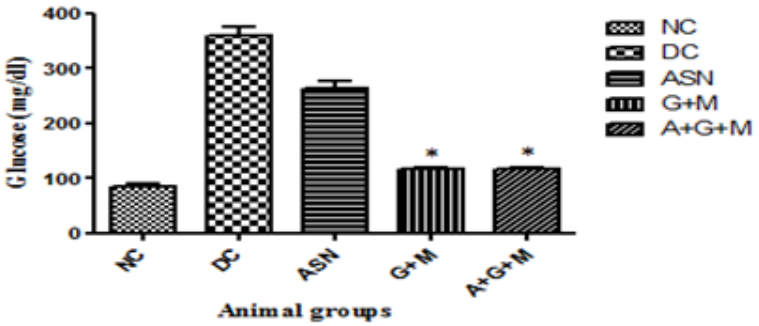
In the streptozotocin induced type 2 diabetic rats, blood glucose levels were significantly raised when compared with normal control group and were considered as diabetic. Treatment (for about 6 weeks) with atorvastatin alone rendered no significant decrease in the blood glucose levels. Treatment with oral hypoglycemic agents (metformin plus glimepiride) and the combination of a atorvastatin with oral hypoglycemic agents (triple therapy), both rendered a significant ($p<0.05$) decrease in the blood glucose levels to a near normal level when compared with diabetic control (Fig. 1).

Effect on serum lipids

In the diabetic rats, there was a significant and pathological abnormality in the levels of serum lipids (LDL, HDL, and triglycerides) when compared with normal control. Treatment with atorvastatin alone significantly ($p<0.05$) decreased the levels of LDL and the

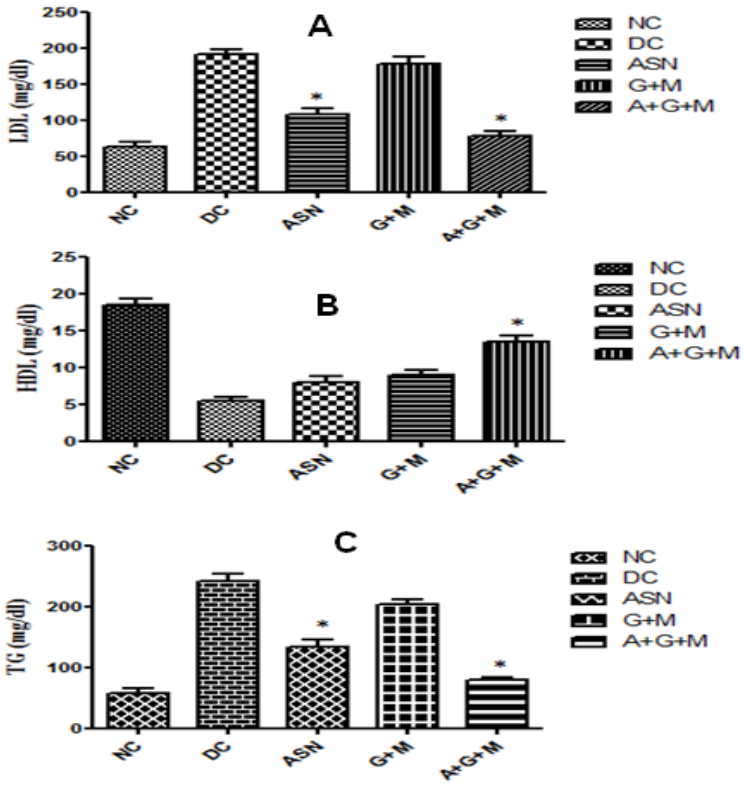
triglycerides, whereas rendered no significant improvement in the levels of HDL when compared with diabetic control. Treatment with oral hypoglycemic agents yielded no significant reversal in the pathologically abnormal levels of serum lipids. Triple therapy significantly ($p<0.05$) improved the levels of HDL, furthermore yielded a significant reduction in the levels of LDL and the triglycerides when compared with diabetic control (Fig. 2).

Fig. 1. Blood glucose (mg/dl) levels of different groups.



Values are presented as mean ± SEM; n=6. Statistical differences between the treated and the diabetic control groups were considered as significant at *p<0.05.
NC= Normal control, DC=Drug Control, ASN=Atorvastatin treated,
G+M= Glimepride and Metformin treated and A+G+M= Triple therapy

Fig. 2. Low density lipoprotein (mg/dl), High density lipoprotein (mg/dl) and Triglyceride (mg/dl) levels of different groups.



A) Low density lipoprotein (mg/dl) levels of different groups
B) High density lipoprotein (mg/dl) levels of different groups
C) Triglyceride (mg/dl) levels of different groups

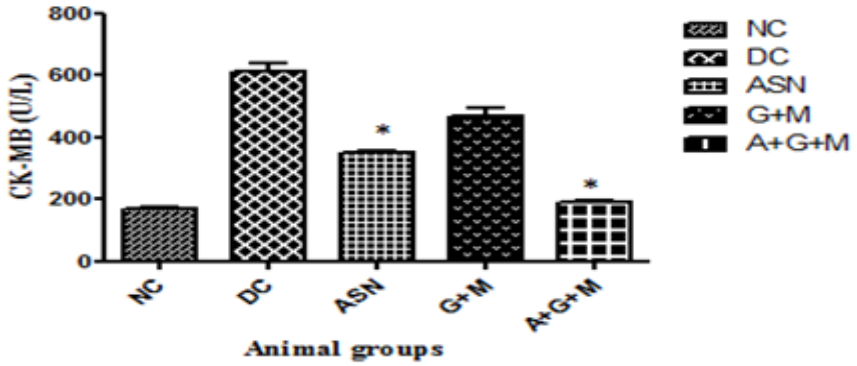
Values are presented as mean ± SEM; n=6. Statistical differences between the treated and the diabetic control groups were considered as significant at *p<0.05.
NC= Normal control, DC=Drug Control, ASN=Atorvastatin treated,
G+M= Glimepride and Metformin treated and A+G+M= Triple therapy

ISSN: 0975-8585

Effect on CK-MB levels

Creatine kinase- MB (CK-MB) levels were significantly raised in the diabetic rats when compared with normal control group. Triple therapy and atorvastatin monotherapy decreased the CK-MB levels significantly ($p<0.05$) to a near normal level when compared with diabetic control. Treatment with oral hypoglycemic agents rendered no significant decrease in this cardiac risk marker (Fig. 3).

Fig. 3. Creatine kinase-MB (U/L) levels of different groups.



Values are presented as mean ± SEM; n=6. Statistical differences between the treated and the diabetic control groups were considered as significant at *$p<0.05$. NC= Normal control, DC=Drug Control, ASN=Atorvastatin treated, G+M= Glimepride and Metformin treated and A+G+M= Triple therapy

Effect on renal parameters

Renal risk markers (creatinine, BUN, and total protein) were found to be pathologically anomalous in the diabetic rats when compared with normal control. Triple therapy, significantly ($p<0.05$) brought down the levels of creatinine to a level, closer to the physiological range. Atorvastatin monotherapy and antidiabetic drug therapy both yielded no significant reversal in the serum creatinine levels. On the other hand, none of the therapies rendered the levels of urea nitrogen to a near normal range. A significant ($p<0.05$) improvement in the total protein levels were found after the stipulated statin monotherapy and triple combination therapy when compared with diabetic control, whereas oral hypoglycemic therapy failed to do so (Fig. 4).

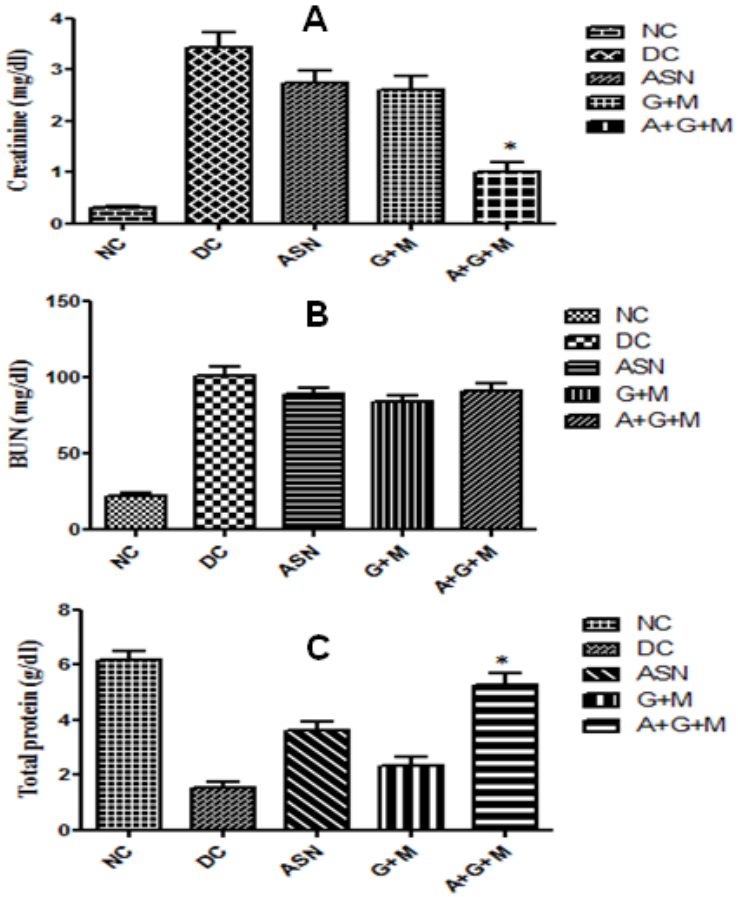
Effect on antioxidant parameters in the brain

Oxidative stress marker, malondialdehyde (MDA) levels were significantly higher and the antioxidant defense markers, catalase (CAT) and glutathione peroxidase (GPx) concentrations were significantly lower in the brain homogenates of streptozotocin induced type 2 diabetic rats when compared with normal control.

Atorvastatin monotherapy, significantly ($p<0.05$) curbed the levels of MDA and augmented the concentration of CAT to an almost physilogical rage, on the otherhand failed to improve GPx concentration, when compared with diabetic control. Antidiabetic drug

therapy found to be insignificant ($p>0.05$) in reversing the abnormality in the concentrations of antioxidant parameters (MDA, CAT and GPx) accompanied by type 2 diabetes, in contrary triple therapy found to be significant ($p<0.05$) in doing so, when compared with diabetic control (Fig. 5).

Fig. 4. Serum creatinine (mg/dl), Blood urea nitrogen (mg/dl) and Total protein (g/dl) levels of different groups.



A) Serum creatinine (mg/dl) levels of different groups
B) Blood urea nitrogen (mg/dl) levels of different groups
C) Total protein (g/dl) levels of different groups
Values are presented as mean ± SEM; n=6. Statistical differences between the treated and the diabetic control groups were considered as significant at *p<0.05.
NC= Normal control, DC=Drug Control, ASN=Atorvastatin treated,
G+M= Glimepride and Metformin treated and A+G+M= Triple therapy

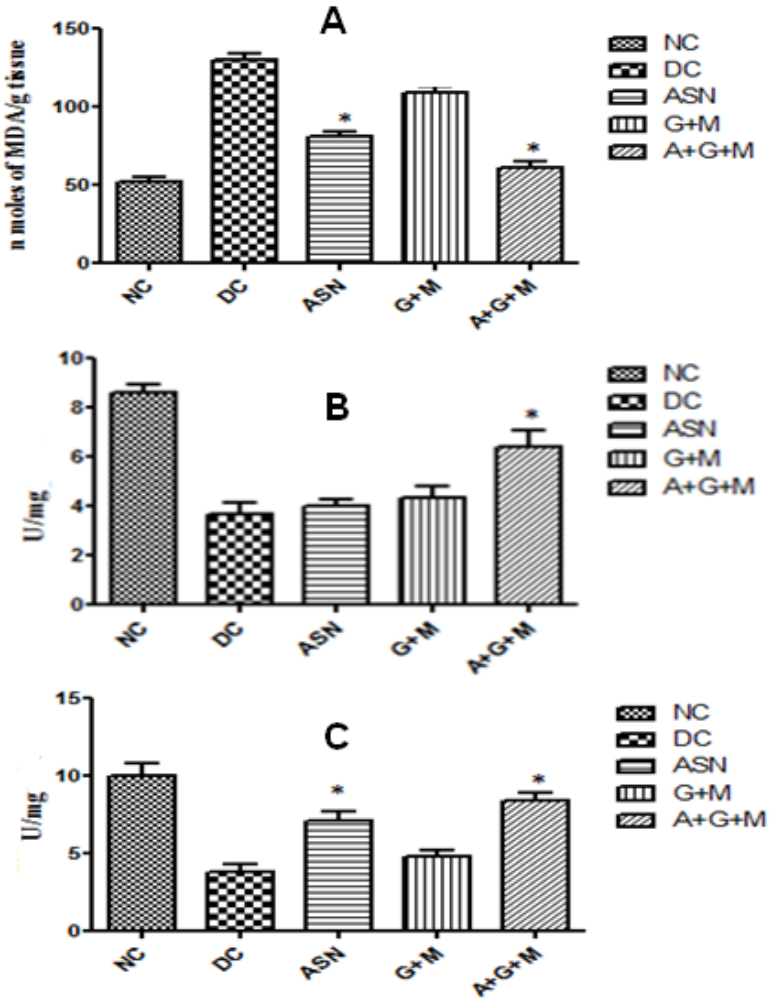
Effect on antioxidant parameters in the kidney

In the kidney homogenates of diabetic rats, there found to be a considerable derailment in the antioxidant parameters (CAT, GPx, and MDA) when compared with normal control. Triple combination therapy and atorvastatin monotherapy significantly ($p<0.05$) reduced the oxidative stress marker (MDA) concentrations whereas oral hypoglycemic therapy failed to do so. Antioxidant defense marker concentrations (CAT and GPx) were significantly ($p<0.05$) improved to a near physiological range by triple therapy when compared with diabetic control. On the other hand, antidiabetic drug therapy and alone

ISSN: 0975-8585

atorvastatin therapy rendered an insignificant increase in the antioxidant defenses in the kidney homogenates when compared with diabetic control (Fig. 6).

Fig. 5. MDA (nmol/g), GPx (U/mg) and CAT (U/mg) levels in brain homogenates of different groups.



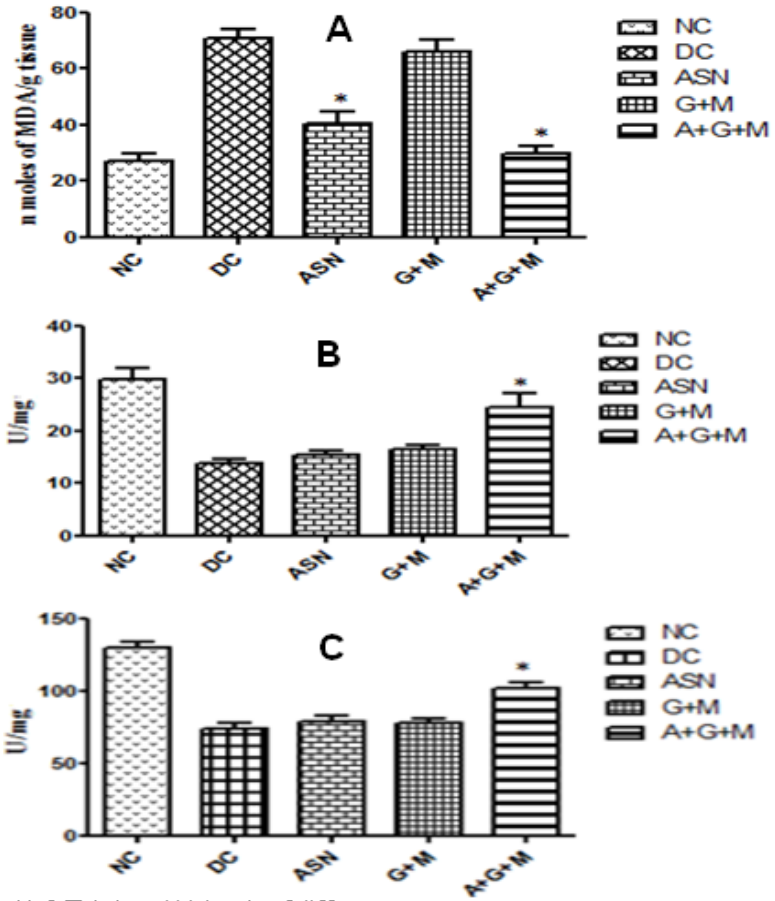
A) MDA (nmol/g) levels of different groups
B) GPx (U/mg) levels of different groups
C) CAT (U/mg) levels of different groups
Values are presented as mean ± SEM; n=6. Statistical differences between the treated and the diabetic control groups were considered as significant at *p<0.05.
NC= Normal control, DC=Drug Control, ASN=Atorvastatin treated,

Effect on nerve fiber function (tail-withdrawal latency)

A significant increase in the tail-withdrawal latency time was observed in the streptozotocin induced type 2 diabetic animals when compared with normal control. Reduction in the tail-withdrawal latency time with atorvastatin monotherapy and with glimepiride plus metformin therapy found to be insignificant, while triple combination therapy significantly ($p<0.05$) decreased the latency time for tail-withdrawal from hot water to a near normal range when compared with diabetic control (Fig. 7).

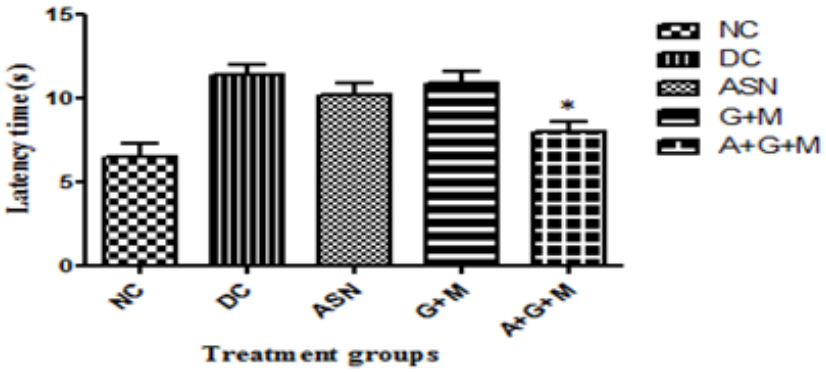
ISSN: 0975-8585

Fig. 6. MDA (nmol/g), GPx (U/mg) and CAT (U/mg) levels in kidney homogenates of different groups.



A) MDA (nmol/g) levels of different groups
B) GPx (U/mg) levels of different groups
C) CAT (U/mg) levels of different groups
Values are presented as mean ± SEM; n=6. Statistical differences between the treated and the diabetic control groups were considered as significant at *p<0.05.
NC= Normal control, DC=Drug Control, ASN=Atorvastatin treated,
G+M= Glimepride and Metformin treated and A+G+M= Triple therapy

Fig. 7. Tail-withdrawal latency time (s) of different groups.



Values are presented as mean ± SEM; n=6. Statistical differences between the treated and the diabetic control groups were considered as significant at *p<0.05.
NC= Normal control, DC=Drug Control, ASN=Atorvastatin treated,
G+M= Glimepride and Metformin treated and A+G+M= Triple therapy

DISCUSSION

This is the first study in which beneficial pleiotropic effects of statins were explored to assess whether they can counter the development and progression of debilitating diabetic complications when combined with oral hypoglycemic agents.

The results of the present study inferred that the treatment of streptozotocin-induced type 2 diabetic Wistar Albino rats with the combination of a statin (atorvastatin) and oral hypoglycemic agents (metformin + glimepiride) provides cardioprotection and alleviates neural and renal complications in addition to better and efficient glycemic control. This triple therapy (atorvastatin + glimepiride + metformin) resulted in efficient glycemic control in the diabetic rats. One of the previous studies involving statins reported that atorvastatin improves glycemic control and insulin sensitivity via the activation of peroxisome proliferator-activatated receptor-γ (PPAR-γ) via 15-deoxy-delta-12, 14-PGJ_2 ($15DPGJ_2$) [13].

This study demonstrated that atorvastatin when combined with oral hypoglycemic agents, alleviates neural complications associated with type 2 diabetes. This was evidenced by better glycemic control along with a significant reduction in the concentration of MDA (biomarker for lipid peroxidation) and a significant rise in the concentrations of antioxidant enzymes (GPx and CAT) in the brain homogenates of diabetic rats treated with triple therapy which indicates minimal damage to the neural cell membranes.

In the literature, it was reported that atorvastatin has antioxidant activity and can reduce oxidative stress [14]. The mechanisms that are attributable to the antioxidant activity of atorvastatin are increase in the bioavailability of nitric oxide (NO), decrease in the lipid peroxidation and the ROS production [15].

This study also demonstrated that triple therapy improves nerve fiber conduction in diabetic rats which was evidenced by decrease in the tail withdrawal latency time (s) when the animals were subjected to tail immersion test (thermal stimulus model to assess small nerve fiber function). Reports suggest that studies on the streptozotocin-induced diabetic rat model, rats with diabetes for 6 weeks had 21.4 and 13.6% deficits in sciatic motor and saphenous sensory nerve conduction velocities, respectively, compared with control animals. Rosuvastatin treatment with 0.3 -20 mg/kg for 2 weeks corrected these deficits in a dose-dependent manner. The conduction velocity effects serve as a measure of large nerve fiber dysfunction. Small fiber system effects were assessed by foot withdrawal latency, using a standard thermal stimulus model. Diabetic rats exhibited thermal hyperalgesia with a 33% reduction in withdrawal latency; this reduction was 86.7% corrected with rosuvastatin 20 mg/ kg treatment, indicating improved function of small nerve fibers. Statin treatment corrects nerve dysfunction in experimental diabetes, acting on both large and small fiber systems. A key mechanism of these beneficial effects appears to be improvement in neural tissue perfusion [16, 17].

In this study, atorvastatin in combination with oral hypoglycemic agents demonstrated a potential cardioprotective action against diabetic dyslipidemia and the consequent atherosclerotic lesions, coronary artery disease (CAD), acute coronary coronary syndrome (ACS) and myocardial infarction (MI). Triple therapy rendered significant changes

in the raised LDL, triglyceride, and creatine kinase-MB concentrations and reduced HDL concentration in the diabetic rats.

The cardioprotective role of triple therapy might be due to effective control on blood glucose levels and lipid concentrations by the combination of metformin with a sulfonylurea in the diabetic rats [18]. In addition to this, beneficial cardiovascular pleiotropic effects of atorvastatin which include improvement of endothelial dysfunction, increased nitric oxide (NO) bioavailability, antioxidant properties, inhibition of inflammatory responses, and stabilization of atherosclerotic plaques, might act in concert with the potent lipid lowering effect of statins to exert early as well as lasting cardiovascular protective effects [19]. In one of the previous studies, it was reported that cardioprotective activity can be assessed by a significant reduction in the levels of creatine kinase-MB (CK-MB) along with significant changes in the other cardiac risk markers and the antioxidant defense markers like MDA and CAT [20].

The results of the present study, obtained by triple therapy to the diabetic animals revealed that this combination mitigates renal anomalies associated with diabetic nephropathy. Significant reduction in the concentration of serum creatinine, and an increase in the total protein content (i.e. decrease in proteinuria) along with better glycemic control were the corresponding findings observed with the triple therapy to the diabetic rats. In addition to this, there were also the findings denoting significant positive changes in the concentrations of antioxidant parameters in the kidney homogenates of diabetic rats after the stipulated period of triple therapy.

The renoprotective role of statin combined with oral hypoglycemics was attributed to strict glycemic control, improvement in the insulin sensitivity, lowered serum lipid content, decreased ROS production and reduced protein overload as well [21]. In the literature, there was an *in vivo* evidence for the assessment of renoprotective effect wherein the renal risk markers were significantly decreased (creatinine, BUN and Proteinuria) along with a significant reduction in the oxidative stress marker (MDA) and a significant rise in the antioxidant defense markers (GSH, CAT) [22].
At the end, triple therapy (atorvastatin in combination with glimepiride plus metformin) improved glycemic control and the antioxidant defenses, enhanced nerve fiber functioning, substantially decreased cardiac risk markers (LDL, triglycerides and CK-MB), and reduced the protein overload thereby alleviated renal damage, in comparison to glimepiride plus metformin therapy in streptozotocin induced type 2 diabetic Wistar Albino rats.

Type 2 diabetic patients are often treated with a combination of antidiabetic agents. The need to use drugs with different and complementary mechanisms of action frequently arises in daily clinical practice. There are several reasons to do this; namely, the disease itself is progressive, with deterioration of glycemic control over time, and monotherapeutic attempts to achieve and maintain glycemic control often fail in the long run[8]. The combination of a sulfonylurea with metformin is commonly used in clinical practice. But when this potent combination is no longer able to prevent the disease progression with mere glycemic control, then the addition of an agent like statin with different mode of action and with reported, beneficial pleiotropic effects may lead to improved metabolic control and alleviated vital organ damage [23].

CONCLUSIONS

In conclusion, atorvastatin demonstrated potential cardioprotective effect in streptozotocin induced type 2 diabetic Wistar Albino rats undergoing treatment with glimepiride plus metformin. Atorvastatin also alleviated neural and renal complications associated with type 2 diabetes when combined with oral hypoglycemic agents. Initial management of diabetes may not necessitate this triple therapy but based on the disease progression, prognostic and diagnostic features, combination of an agent like statin with oral hypoglycemic agents could be an intervention to curb vital organ damage associated with type 2 diabetes. Further studies are necessary, first, to warrant whether this triple combination therapy provides protection against any other diabetic anomalies in addition to the above mentioned complications, second, to provide insights into explicit molecular mechanisms underlying the outcomes of this study.

REFERENCES

[1] Khaled A, Ikram S. Biomed Res 2010; 21: 147-155.
[2] Grundy SM, Benjamin IJ, Burke GL. Circulation 1999; 100: 1134–1146.
[3] Collins R, Armitage J, Parish S, Sleigh P, Peto R. Lancet 2003; 361: 2005–2016.
[4] American Diabetes Association. Diabetes Care 2004; 27: 68–71
[5] Yagihashi S. Diabetes Metab Rev 1995; 11: 193-225.
[6] Watts GF, O'Brien SF, Silvester W, Millar JA. Clin Sci (Lond) 1996; 91: 567-573.
[7] Peterson JC, Adler S, Burkart JM, Greene T, Herbert LA, Hunsicker LG, King AJ, Klahr S, Massry SG, Seifter JL. Ann Intern Med 1995; 123: 754–762.
[8] Turner RC, Cull CA, Frighi V, Holman RR. JAMA 1999; 281: 2005–2012.
[9] Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Margaret W, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. J Clin Invest 2001; 108: 1167-74.
[10] Dills DG, Schneider J. Horm Metab Res 1996; 28: 426-429.
[11] Tandon V, Bano G, Khajuria V, Parihar A, Gupta S. Ind J Pharmacol 2005; 37: 77-85.
[12] Abeeleh M.A, Ismail ZB, Alzaben KR, Abu-Halaweh SA, Al-Essa MK, Abuabeeleh J, Alsmady MM. Eur J Sci Res 2009; 32: 398-402.
[13] Freeman DJ, Norrie J, Sattar N, Neely RDG, Cobbe SM, Ford I, Isles C, Lorimer AR, Macfarlane PW, McKillop JH, Packard CJ, Shepherd J, Gaw A. Circulation 2001; 103: 357–362.
[14] Buyukhatipoglu H, Sezen Y, Ali Yildiz A, Guntekin U, Bas M, Polat M, Demirbag R, Taskin A, Celik H, Aksoy N. Clin Invest Med 2010; 33: 313-320.
[15] Wassmann S, Lauf U, Baumer AT, Muller K, Albory K, Linz W, Itter G, Rosen R, Bohm M, Nickenig G. Hypertension 2001; 37: 1450-1457.
[16] Cameron NE, Cotter MA. Diab Res Clin Pract 1999; 45: 137-146.
[17] Cameron NE, Eaton SE, Cotter MA, Tesfaye S. Diabetol 2001; 44: 1973-1988.
[18] DeFronzo RA, Goodman AM. New Engl J Med 1995; 333: 541–549.
[19] Davignon J. Circulation 2004; 109: 39-43.
[20] Momin FN, Kalai BR, Shikalgar TS, Naikwade NS. Ind J Pharmacol 2012; 44: 178-183.
[21] Buemi M, Allegra A, Corica F, Aloisi C, Giacobbe M, Pettinato G, Corsonello A, Senatore M, Frisina N. Clin Pharmacol Ther 2000; 67: 427-431.

[22] Hassan HA, El-Agmy SM, Gaur RL, Fernando A, Raj HG, Ouhtit A. Int J Biol Sci 2009; 5: 249-255.
[23] Mathews DR, Cull CA, Stratton IM, Holman RR, Turner RC. Diabet Med 1998; 15, 297–303.