Research Article

ROLE OF NITRIC OXIDE DONOR AND PHOSPHODIESTERASE-II INHIBITOR IN A STREPTOZOTOCIN INDUCED DIABETIC NEPHROPATHY IN RATS

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ABSTRACT

The aim of the present study was to investigate the role of Sodium Nitroprusside (SNP), a nitric oxide donor alone or combination with erythro-9-(2-hydroxy-3-nonyl) adenine, (EHNA), a Phosphodiesterase-II specific inhibitor in the reduction of various markers of nephropathy, reactive oxygen species (ROS) and endogenous antioxidants enzymes activity. The serum creatinine, blood urea nitrogen (BUN), proteinuria, cholesterol level, absolute kidney weight, kidney hypertrophy, renal collagen content, nitrite/ nitrate concentration, ROS were assessed by estimating Thiobarbituric Acid Reactive Substances (TABRS) level and endogenous antioxidants enzymatic activities (SOD, Catalase and GSH) were examined in 80 male Wistar rats that were allocated in ten groups (n=8). The experimental protocol includes normal and diabetic control groups, SNP per se, Aldosterone per se group, SNP-1mg, SNP-2mg treated diabetic rats alone or in combination with EHNA and Aldosterone for 2 weeks after 6 weeks of STZ administration. Significant increase in serum creatinine, BUN, proteinuria, cholesterol level, absolute kidney weight, kidney hypertrophy, renal collagen content, TBARS and significant decrease in serum nitrite/nitrate, endogenous antioxidant enzymes activity was detected in serum and kidney homogenate in the diabetic groups. Intraperitoneal administration of SNP alone and in combination with EHNA groups was found statistically significant for reducing the markers of nephropathy, ROS and antioxidants activities. There was statistical difference observed when Aldosterone was administered in combination with SNP 1 & 2 mg in diabetic rats. Diabetes induced renal injury was found to be reduced by the intraperitoneal administration of SNP in dose dependent manner, even in the presence of EHNA. However beneficial effects were attenuated when Aldosterone was administered in combination with SNP in diabetic rats.

Keywords: Sodium Nitroprusside, Thiobarbituric Acid Reactive Substances, hypertrophy

INTRODUCTION

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease in the Western hemisphere. Endothelial dysfunction is the central pathophysiologic denominator for all cardiovascular complications of diabetes including nephropathy[1]. Endothelial cells maintain vascular function and homeostasis by generating paracrine factors that regulate vascular tone, preventing coagulation and platelet aggregation, inhibiting adhesion of leukocytes, and limiting proliferation of vascular smooth muscle cells[2].

Further, it was shown that nitric oxide (NO) produced by endothelial cells through the endothelial nitric oxide synthase (eNOS) plays a major role for many of these endothelial functions[3] and that decreased NO production and bioavailability largely contribute to endothelial dysfunction in diabetes[4,5]. NO, a potent endogenous vasodilator and is a key regulatory molecule with extensive metabolic, vascular, and cellular effects[6]. NO plays a critical role in regulation of renal hemodynamics[7]. The kidney plays an important role in body NO homeostasis. *Iqbal et al.* have shown that exogenous NO donor administration, prevents the oxidative stress, and restores the renal functions[8].

Sodium Nitroprusside (SNP), an NO donor, has vasodilator effects. It is used for the treatment of myocardial infarction and pulmonary hypertension. NO donor activates guanylate cyclase in vascular smooth muscle and increases intracellular production of cGMP. cGMP activates protein kinase G which activates phosphatases which inactivate myosin light chains[9]. Myosin light chains are involved in muscle contraction. The end result is vascular smooth muscle relaxation, which allow vessels to dilate[9]. This mechanism is similar to that of Phosphodiesterase 5 (PDE5) inhibitors which elevate cGMP concentration by inhibiting its degradation by PDE5[10].

The purpose of the present study was to investigate whether NO donor and Phosphodiesterase-II inhibitor can attenuate DN in Streptozotocin (STZ)-induced diabetic rats.

MATERIAL AND METHODS
Animals

Male, Wistar rats with an initial body weight of 180-220g were used in this study. Animal care and treatment was conducted in conformity with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India and approved by Institutional Animal Ethical Committee [MMCP-IAEC-17-05]. All animals were housed in standard light/dark cycle with free access to standard diet and tap water ad libitum. The animals were housed in metabolic cages. A 24-h urine collection was obtained from each rat for laboratory investigations.

Experimental design

The estimation Kits for serum glucose, serum creatinine, serum cholesterol, proteins in urine and blood urea nitrogen (BUN) were obtained from Transasia Bio-Medicals Ltd., Baddi, India. All other chemicals used in present study were of analytical grade.

Induction and assessment of diabetes

Wistar rats (180-220g) were used in the present study. Experimental diabetes was induced with single dose of STZ 50mg/kg, i.p. dissolved in ice cold in 0.1 M citrate buffer pH 4.5[11]. Rats having a blood glucose level of 200 mg/dl at 1 week after STZ injection were considered as diabetic and induction was considered successful. Blood samples were obtained from retro-orbital sinus and serum glucose level were determined by glucose oxidase–peroxidase method[12] using commercially available kit (Transasia Bio-Medicals Ltd., Baddi, India).

Preparation of kidney homogenate

Immediately after sacrifice, both the kidneys were dissected and rinsed with ice cold isotonic saline and weighed. The kidney was than minced and a homogenate (10% w/v) was prepared in chilled 1.15% KCL. The homogenate was used for estimating kidney antioxidant and oxidative stress parameters.

Assessment of renal function measurement

Serum creatinine, BUN and protein in urine level were determined in all serum samples by using standard diagnostic kits (Transasia Bio-Medicals Ltd., Baddi, India).

Assessment of serum cholesterol

The serum cholesterol was estimated by blood samples collected by retero orbital sinus by cholesterol oxidase peroxidase method[13] using commercially available kit (Transasia Bio-Medicals Ltd., Baddi, India).

Assessment of renal collagen content

Total renal cortical collagen content was determined by analysis of hydroxyproline content[14].

Estimation of serum nitrite and nitrate levels

Nitrite and nitrate are the primary oxidation products of NO subsequent to reaction with oxygen and, therefore, the nitrite/nitrate concentration in serum was used as an indirect measure of NO synthesis. Quantitation of nitrate and nitrite was based on the Griess reaction, in which a chromophore with a strong absorbance at 550 nm is formed by reaction of nitrite with a mixture of naphthylethylenediamine and sulfanilamide. The nitrate was reduced to nitrite by 30 min incubation with nitrate reductase in the presence of nicotinamide adenine dinucleotide 3-phosphate. Total nitrite/nitrate concentration was calculated by using standard sodium nitrite prepared in K-H buffer. Results were expressed as micromoles per liter[15].

Assessment of renal oxidative stress

The development of oxidative stress in the kidney was assessed by estimating renal Thiobarbituric Acid Reactive Substances (TBARS)[16] and reduced form glutathione (GSH)[17] according to the method described by Ohkawa (1979) and Ellman (1959).

Kidney antioxidant parameters

Antioxidant enzymes activities in the kidney were assessed by estimating superoxide dismutase (SOD)[18] and catalase (CAT)[19] activities in kidney homogenate according to procedure published by Misra & Fradovich (1972) and Aebi (1984).

Experimental procedure (n = 8)

Eighty rats were allocated to following experimental groups, each group consisting of eight animals.
Group I (Normal control): Rats were maintained on standard food and water regimen and no treatment was given.
Group II (SNP in normal rats): Rats were administered SNP high dose (2 mg/kg, i.p.) in 0.9% normal saline for 2 weeks.
Group III (Aldosterone in normal rats): Rats were administered Aldosterone (0.8 µg/100g, i.p.) in 0.9% normal saline for 2 weeks.
Group IV (Diabetic Control): Normal rats were injected STZ (50 mg/kg, i.p. once) dissolved in 0.1 M citrate buffer of pH 4.5
Group V (SNP-1mg treated diabetic rats): Diabetic rats were treated with SNP (1mg/kg, i.p.) in 0.9% normal saline for 2 weeks, after 6 weeks of STZ administration.
Group VI (SNP-2mg treated diabetic rats): Diabetic rats were treated with SNP (2mg/kg, i.p.) in 0.9% normal saline for 2 weeks, after 6 weeks of STZ administration.
Group VII (SNP-1 mg + EHNA hydrochloride treated diabetic rats): Diabetic rats were treated with SNP (1 mg/kg, i.p.) in combination with EHNA hydrochloride (3mg/kg, i.p.) in 0.9% normal saline for 2 weeks, after 6 weeks of STZ administration.
Group VIII (SNP-2 mg + EHNA hydrochloride treated diabetic rats): Diabetic rats were treated with SNP (2 mg/kg, i.p.) in combination with EHNA hydrochloride (3mg/kg, i.p.) in 0.9% normal saline for 2 weeks, after 6 weeks of STZ administration.
Group IX (SNP-1 mg + Aldosterone treated diabetic rats): Diabetic rats were treated with SNP (1 mg/kg, i.p.) in combination with Aldosterone (0.8 µg/ 100g, i.p.) in 0.9% normal saline for 2 weeks, after 6 weeks of STZ administration.
Group X (SNP-2 mg + Aldosterone treated diabetic rats): Diabetic rats were treated with SNP (2 mg/kg, i.p.) in combination with Aldosterone (0.8 µg/ 100g, i.p.) in 0.9% normal saline for 2 weeks, after 6 weeks of STZ administration.

STATISTICAL ANALYSIS

All results were statistically analyzed using Graph Pad Prism version 5.01 (Graph pad software, INC, La Jolla, CA, USA) and expressed as the mean ± standard deviation. Observed data were analyzed using two-way analysis of variance followed by Dunnett's multiple comparison tests for comparing means from different treatment groups. A level of $P < 0.05$ was considered statistically significant.

RESULTS

Results of our study shows that, administration of SNP (2 mg/kg, i.p., 2 weeks) at high doses or Aldosterone (0.8 μg/100g, i.p.) to normal rats did not produce any significant per se effect on various parameters in the present study. SNP induced dose dependent effects on various markers of DN, reactive oxygen species (ROS) accessed by estimating TBARS concentration, endogenous antioxidants (SOD, CAT and GSH) level. In addition dose-dependent effects of SNP were unaltered when administered along with EHNA, PDE-2 specific inhibitor. However administration of Aldosterone significantly attenuated the SNP induced ameliorative effects in diabetic rats.

All the parameters were assessed at the end of two weeks in normal and diabetic rats with or without treatment. Less than 10% of mortality rate was observed in diabetic rats with or without drug treatments.

Effect of Pharmacological Interventions on Body Weight

Administration of STZ significantly (p<0.05) decreased the body weight in diabetic rats when compared with normal control, SNP per se and Aldosterone per se groups. Two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) significantly raised the body weight in dose dependent manner as compared to diabetic control group. However two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) in combination with EHNA, a PDE-2 specific inhibitor (3 mg/kg i.p., 2 weeks) did not significantly alter the effect produced by SNP at (1 mg/kg and 2 mg/kg, i.p.,) in diabetic rats. Moreover, administration of Aldosterone (0.8μg/100g) in combination with SNP (1 mg/kg and 2 mg/kg, i.p.) for two weeks significantly attenuated SNP induced increase in body weight in diabetic rats (Figure 1).

Effect of pharmacological interventions on serum glucose

The marked increase in serum concentration of glucose was noted in diabetic rats when compared with normal rats. Two weeks treatment with SNP at (1 mg/kg and 2 mg/kg, i.p.,) alone or in combination with EHNA (3 mg/kg i.p.,) or in combination with Aldosterone (0.8μg/ 100g) did not significantly alter the serum glucose level as compared to diabetic control group (Figure 2).

Effect of pharmacological interventions on urine output

Volume of urine output was noted to be significantly increased (P<0.05) in diabetic rats when compared with normal rats. Two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) significantly reduced the urine volume in dose dependent manner as compared to diabetic control group. However two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) in combination with EHNA, a PDE-2 specific inhibitor (3 mg/kg i.p., 2 weeks) did not significantly alter the effect produced by SNP at (1 mg/kg and 2 mg/kg, i.p.,) in diabetic rats. Moreover, administration of Aldosterone (0.8μg/100g) in combination with SNP (1 mg/kg and 2 mg/kg, i.p.) for two weeks significantly attenuated SNP induced decrease in urine output in diabetic rats (Figure 3).

Effect of pharmacological interventions on serum creatinine, blood urea nitrogen and proteinuria

The concentrations of serum creatinine, BUN, and proteinuria were noted to be markedly increased (P<0.05) in diabetic rats after six weeks of STZ administration when compared with normal rats. Two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) significantly reduced the serum creatinine, BUN, and proteinurea in dose dependent manner as compared to diabetic control group. However two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) in combination with EHNA, a PDE-2 specific inhibitor (3 mg/kg i.p., 2 weeks) did not significantly alter the effect produced by SNP at (1 mg/kg and 2 mg/kg, i.p.,) in diabetic rats. Moreover, administration of Aldosterone (0.8μg/100g) in combination with SNP (1 mg/kg and 2 mg/kg, i.p.) for two weeks significantly attenuated SNP induced decrease in serum creatinine, BUN, and proteinuria in diabetic rats (Figure 4, 5 and 6).

Effect of pharmacological interventions on serum cholesterol

A significant increase (P<0.05) in serum cholesterol was noted in diabetic rats when compared with normal rats. Two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) significantly reduced the serum cholesterol level in dose dependent manner as compared to diabetic control group. However two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) in combination with EHNA, a PDE-2 specific inhibitor (3 mg/kg i.p., 2 weeks) did not significantly alter the effect produced by SNP at (1 mg/kg and 2 mg/kg, i.p.,) in diabetic rats. Moreover, administration of Aldosterone (0.8μg/100g) in combination with SNP (1 mg/kg and 2 mg/kg, i.p.) for two weeks significantly attenuated SNP induced decrease in serum cholesterol level in diabetic rats (Figure 7).

Effect of pharmacological interventions on absolute kidney weight and kidney hypertrophy (kidney weight/ body weight %)

The absolute kidney weight and kidney weight/ body weight (%) in diabetic rats were significantly (P<0.05) increased as compared to normal control group. Two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) significantly reduced the absolute kidney weight and kidney weight/ body weight (%) in dose dependent manner as compared to diabetic control group. However two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) in combination with EHNA, a PDE-2 specific inhibitor (3 mg/kg i.p., 2 weeks) did not significantly alter the effects produced by SNP at (1 mg/kg and 2 mg/kg, i.p.,) in diabetic rats. Moreover, administration of Aldosterone (0.8μg/100g) in combination with SNP (1 mg/kg and 2 mg/kg, i.p.) for two weeks significantly attenuated SNP induced decrease in absolute kidney weight and kidney weight/ body weight (%) in diabetic rats (Figure 8 and 9).

Effect of pharmacological interventions on renal collagen content

A significant increase (P<0.05) in renal cortical collagen content was noted in diabetic rats when compared with normal rats. Two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) significantly reduced the renal cortical collagen content in dose dependent manner as compared to diabetic control group. However two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) in combination with EHNA, a PDE-2 specific inhibitor (3 mg/kg i.p., 2 weeks) did not significantly alter the effects produced by SNP at (1 mg/kg and 2 mg/kg, i.p.,) in diabetic rats. Moreover, administration of Aldosterone (0.8μg/100g) in combination with SNP (1 mg/kg and 2 mg/kg, i.p.) for two weeks significantly attenuated SNP induced decrease in renal cortical collagen content in diabetic rats (Figure 10).

Effect of pharmacological interventions on Serum Nitrite/Nitrate concentration

Diabetic rats 6 weeks after STZ administration showed marked decrease (P<0.05) in serum concentration of nitrite/nitrate as compared to normal rats. Two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) significantly increased the serum nitrite/nitrate level in dose dependent manner as compared to diabetic control group. However two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) in combination with EHNA, a PDE-2 specific inhibitor (3 mg/kg i.p., 2 weeks) did not significantly alter the effects produced by SNP at (1 mg/kg and 2 mg/kg, i.p.,) in diabetic rats.

Moreover, administration of Aldosterone (0.8µg/100g) in combination with SNP (1 mg/kg and 2 mg/kg, i.p.) for two weeks significantly attenuated SNP induced increase in serum nitrite/nitrate level in diabetic rats (Figure 11).

Effect of pharmacological interventions on renal oxidative stress

A significant increase (P<0.05) in TBARS concentration was noted in diabetic rats when compared with normal rats. Two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) significantly decreased the diabetes induced increase in TBARS concentration in dose dependent manner as compared to diabetic control group. However two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) in combination with EHNA, a PDE-2 specific inhibitor (3 mg/kg i.p., 2 weeks) did not significantly alter the effects produced by SNP at (1 mg/kg and 2 mg/kg, i.p.,) in diabetic rats.

Moreover, administration of Aldosterone (0.8µg/100g) in combination with SNP (1 mg/kg and 2 mg/kg, i.p.) for two weeks significantly attenuated SNP induced decrease in TBARS concentration in diabetic rats (Figure 12).

Effect of pharmacological interventions on renal glutathione

A significant decrease (P<0.05) in GSH level was noted in diabetic rats when compared with normal rats. Two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) significantly increased the diabetes induced decrease in GSH level in dose dependent manner as compared to diabetic control group. However two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) in combination with EHNA, a PDE-2 specific inhibitor (3 mg/kg i.p., 2 weeks) did not significantly alter the effects produced by SNP at (1 mg/kg and 2 mg/kg, i.p.,) in diabetic rats. Moreover, administration of Aldosterone (0.8µg/100g) in combination with SNP (1 mg/kg and 2 mg/kg, i.p.) for two weeks significantly attenuated SNP induced increase in GSH level in diabetic rats (Figure 13).

Effect of pharmacological interventions on antioxidant enzymes activities

A significant decrease (P<0.05) in SOD and CAT activity was noted in diabetic rats when compared with normal rats. Two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) significantly increased the SOD and CAT activity in dose dependent manner as compared to diabetic control group. However two weeks treatment with SNP (1 mg/kg and 2 mg/kg, i.p.) in combination with EHNA, a PDE-2 specific inhibitor (3 mg/kg i.p., 2 weeks) did not significantly alter the effects produced by SNP at (1 mg/kg and 2 mg/kg, i.p.,) in diabetic rats. Moreover, administration of Aldosterone (0.8µg/100g) in combination with SNP (1 mg/kg and 2 mg/kg, i.p.) for two weeks significantly attenuated SNP induced increase in SOD and CAT activity in diabetic rats (Figure 14 and 15).

DISCUSSION

Diabetes mellitus (DM) is a complex metabolic syndrome characterized by absolute insulin deficiency or development of insulin resistance that leads to hyperglycemia and an altered glucose, fat and protein metabolism[20]. DN is a microvascular complication and leading cause of morbidity and mortality[21]. In the present study, six weeks after STZ administration significantly increased the proteinuria, urine level, cholesterol level, absolute kidney weight, kidney hypertrophy, renal collagen content, ROS and decline nitrite/ nitrate concentration, SOD, CAT and GSH activity were noted. The concentration of serum nitrite/ nitrate concentration was noted to be an index of vascular endothelial dysfunction (VED)[22,23]. A strong co-relation has been reported between reduced NO and DN. Antioxidant and vasodilatory property of SNP have been reported. Hence the present study was designed to evaluate the effects of SNP in DN in combination of PDE-2 inhibitor. ROS were assessed by estimating TABRS level, found to be markedly elevated in diabetic rats. ROS is considered as a critical marker of DN by decreasing NO level and subsequently cause renal and VED. The findings of our study revealed that treatment with SNP has significantly attenuated the diabetes induced increase in markers of DN in diabetic groups. Two weeks treatment with SNP significantly attenuated the diabetes induced increase in proteinuria, urine level, cholesterol level, absolute kidney weight, kidney hypertrophy, renal collagen content, ROS and declined nitrite/ nitrate concentration and endogenous enzymatic activities. In the present study, SNP has prevented the hyperglycemia induced increase in markers of DN in diabetic rats where it shows its nephroprotective role. However, two weeks administration PDE-2 inhibitor, EHNA in combination with SNP did not altered the effect significantly whereas significant alternations were observed when Aldosterone administered in combination with SNP in diabetic rats.

In our study, STZ induced diabetic rats successfully developed DN which was confirmed by increase in proteinuria, urine level, cholesterol level, absolute kidney weight, kidney hypertrophy, renal collagen content, ROS and reduced nitrite/ nitrate concentration and endogenous enzymes SOD, CAT and GSH activity. However treatment with SNP showed a significant decrease in proteinuria, urine volume, cholesterol level, absolute kidney weight, kidney hypertrophy, renal collagen content, ROS and increased nitrite/ nitrate concentration and improved endogenous enzymes activities. Our results are in agreement with reports from other laboratories. It has been reported that protective effect of SNP is due to its NO-donating property[24] as NO play an important role in maintaining normal functioning of kidney.

ROS plays a crucial role in the pathogenesis of diabetic complications, including DN. Hyperglycemia-induced activation of several pathways results in the excessive formation of ROS that is toxic to the cell. They also interact with the lipid bilayer and produce lipid peroxidation products which further release free radicals and damage to renal tree. SOD, CAT, and GSH are responsible for the detoxification of the ROS[25].

In our study, the ability of SNP to increase renal antioxidant enzyme level is in line with the finding of other laboratories and it has been reported that SNP restored the depleted renal antioxidant enzyme level. However, the beneficial antioxidant effect of SNP is due to the decreasing levels of pro-inflammatory mediators, which increase oxidative stress and the severity of inflammation process[26].
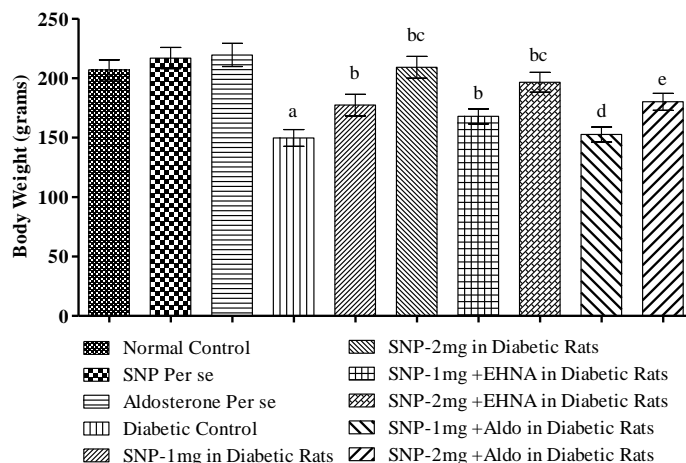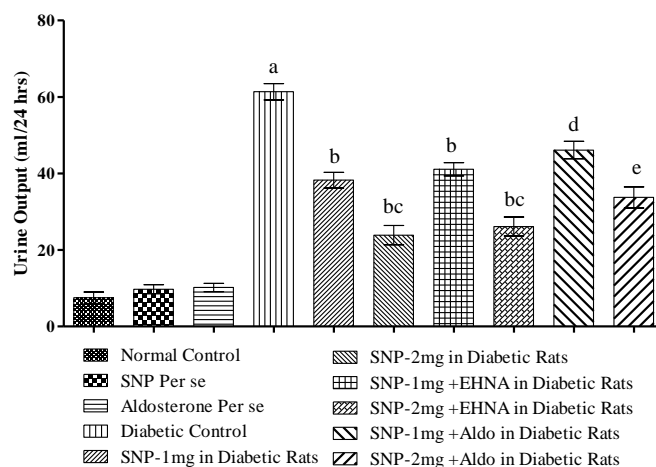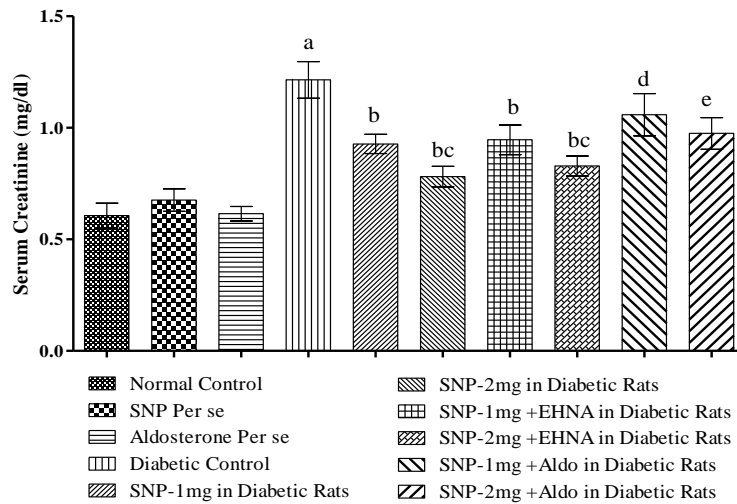
Figure 1: Effect of various pharmacological interventions on Body weight
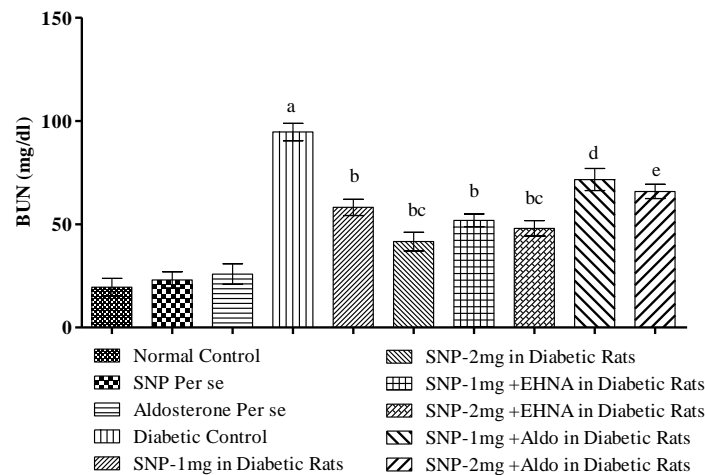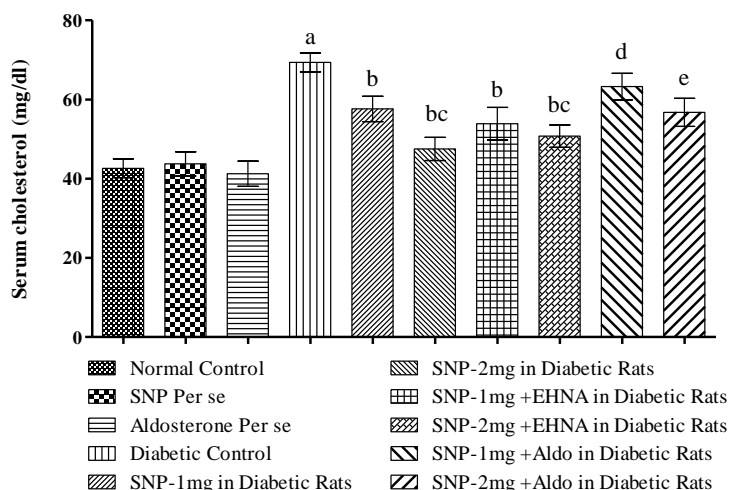Values are expressed as Mean $\pm$ SD, [a] = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*; [b] = p < 0.05 vs diabetic control; [c] = p< 0.05 vs SNP-1 mg treated diabetic rats; [d] = p<0.05 vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e] = p<0.05 vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.
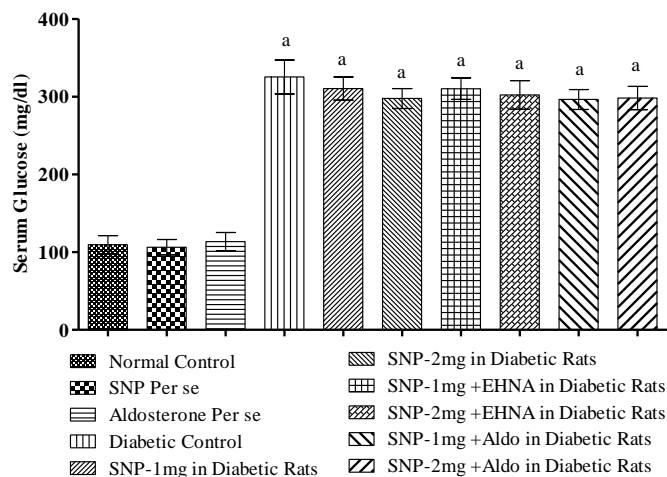
Figure 2: Effect of various pharmacological interventions on Serum Glucose
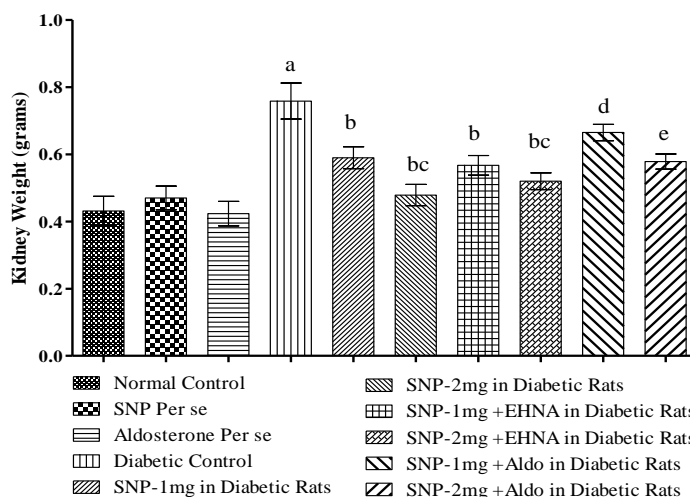Values are expressed as Mean $\pm$ SD, [a] = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*.

Figure 3: Effect of various pharmacological interventions on Urine output
Values are expressed as Mean $\pm$ SD, [a] = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*; [b] = p < 0.05 vs diabetic control; [c] = p< 0.05 vs SNP-1 mg treated diabetic rats; [d] = p<0.05 vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e] = p<0.05 vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.

Figure 4: Effect of various pharmacological interventions on Creatinine

Values are expressed as Mean $\pm$ SD, [a] = $p < 0.05$ vs normal control, SNP *perse* and Aldosterone *perse*; [b] = $p < 0.05$ vs diabetic control; [c] = $p < 0.05$ vs SNP-1 mg treated diabetic rats; [d] = $p<0.05$ vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e] = $p<0.05$ vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.

Figure 5: Effect of various pharmacological interventions on Blood Urea Nitrogen

Values are expressed as Mean $\pm$ SD, [a] = $p < 0.05$ vs normal control, SNP *perse* and Aldosterone *perse*; [b] = $p < 0.05$ vs diabetic control; [c] = $p< 0.05$ vs SNP-1 mg treated diabetic rats; [d] = $p<0.05$ vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e] = $p<0.05$ vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.
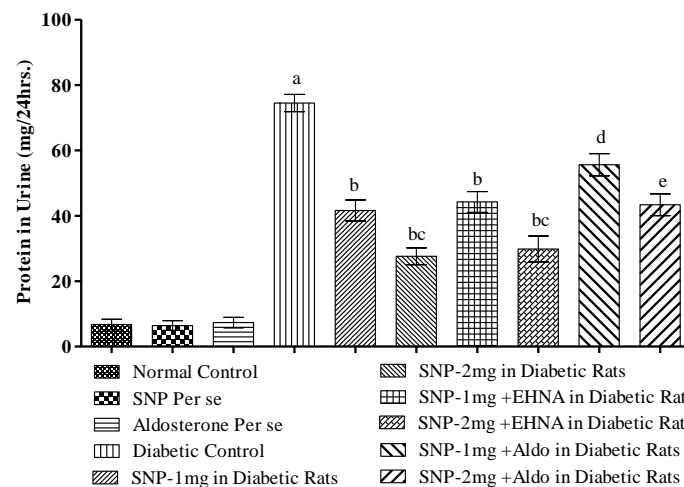
Figure 6: Effect of various pharmacological interventions on urinary protein

Values are expressed as Mean $\pm$ SD, [a] = $p < 0.05$ vs normal control, SNP *perse* and Aldosterone *perse*; [b] = $p < 0.05$ vs diabetic control; [c] = $p< 0.05$ vs SNP-1 mg treated diabetic rats; [d] = $p<0.05$ vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e] = $p<0.05$ vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.
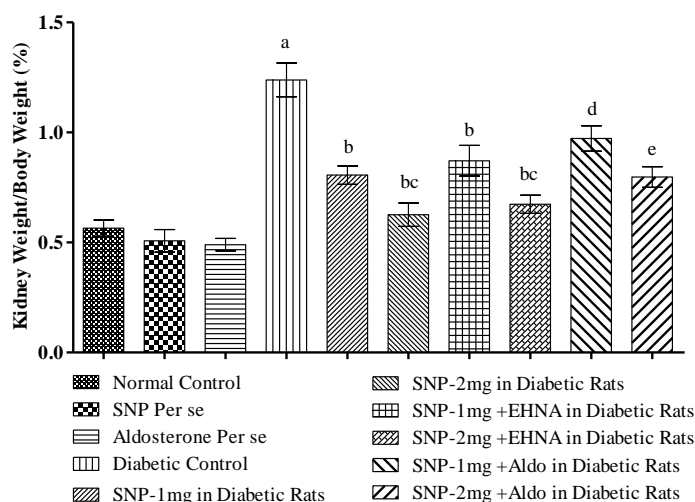
Figure 7: Effect of various pharmacological interventions on serum cholesterol
Values are expressed as Mean $\pm$ SD, [a] = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*; [b] = p < 0.05 vs diabetic control; [c] = p< 0.05 vs SNP-1 mg treated diabetic rats; [d] = p<0.05 vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e]= p<0.05 vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.

Figure 8: Effect of various pharmacological interventions on absolute Kidney weight
Values are expressed as Mean $\pm$ SD, [a] = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*; [b] = p < 0.05 vs diabetic control; [c] = p< 0.05 vs SNP-1 mg treated diabetic rats; [d] = p<0.05 vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e]= p<0.05 vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.

Figure 9: Effect of various pharmacological interventions on kidney hypertrophy (Kidney weight/body weight)
Values are expressed as Mean $\pm$ SD, [a] = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*; [b] = p < 0.05 vs diabetic control; [c] = p< 0.05 vs SNP-1 mg treated diabetic rats; [d] = p<0.05 vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e]= p<0.05 vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.
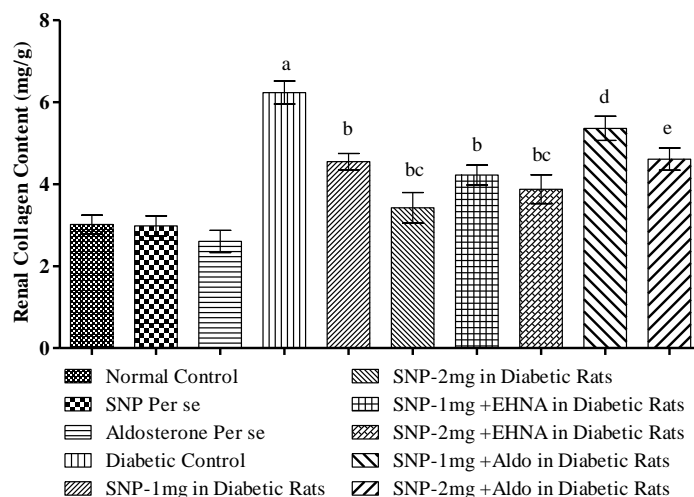
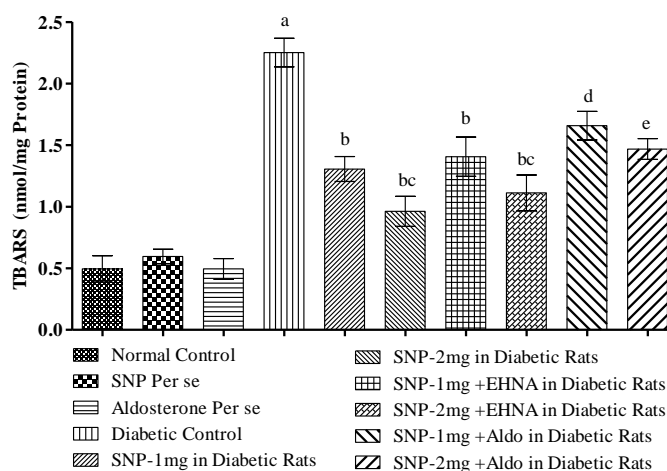**Figure 10: Effect of various pharmacological interventions on renal collagen content**
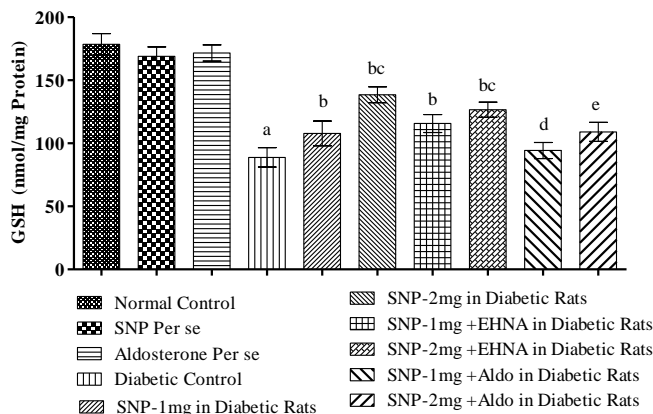
Values are expressed as Mean $\pm$ SD, [a] = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*; [b] = p < 0.05 vs diabetic control; [c] = p< 0.05 vs SNP-1 mg treated diabetic rats; [d]= p<0.05 vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e]= p<0.05 vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.
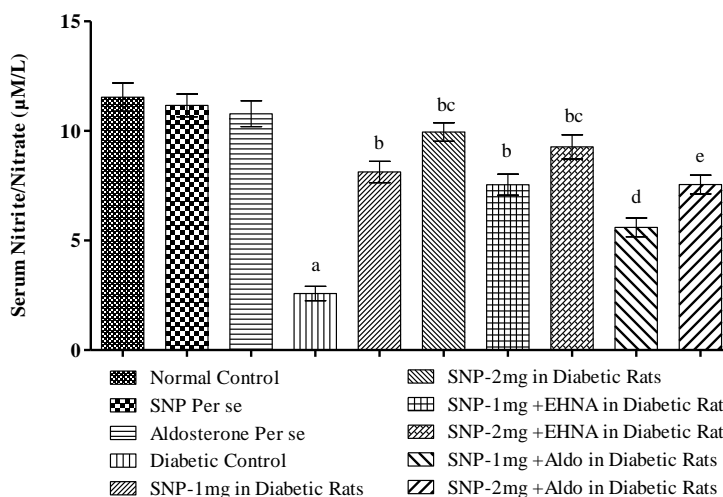
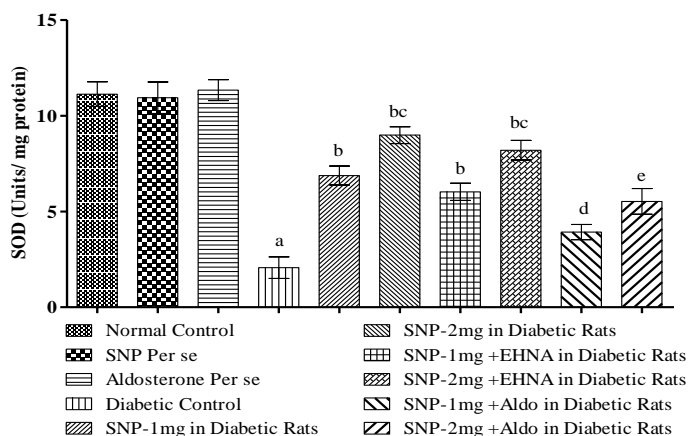**Figure 11: Effect of various pharmacological interventions on serum nitrite/nitrate**

Values are expressed as Mean + SD, a = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*; [b] = p < 0.05 vs diabetic control; [c] = p< 0.05 vs SNP-1 mg treated diabetic rats; [d]= p<0.05 vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e]= p<0.05 vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.

Figure 12: Effect of various pharmacological interventions on TBARS

Values are expressed as Mean $\pm$ SD, [a] = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*; [c] = p< 0.05 vs SNP-1 mg treated diabetic rats; [d]= p<0.05 vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e]= p<0.05 vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.
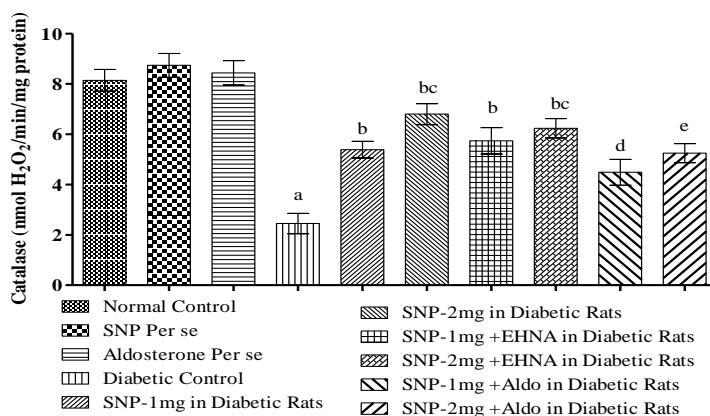
Figure 13: Effect of various pharmacological interventions on GSH

Values are expressed as Mean $\pm$ SD, [a] = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*; [b] = p < 0.05 vs diabetic control; [c] = p< 0.05 vs SNP-1 mg treated diabetic rats; [d] = p<0.05 vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e] = p<0.05 vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.

Figure 14: Effect of various pharmacological interventions on SOD

Values are expressed as Mean + SD, a = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*; [b] = p < 0.05 vs diabetic control; [c] = p< 0.05 vs SNP-1 mg treated diabetic rats; [d] = p<0.05 vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e] = p<0.05 vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.

Figure 15: Effect of various pharmacological interventions on Catalase

Values are expressed as Mean $\pm$ SD, [a] = p < 0.05 vs normal control, SNP *perse* and Aldosterone *perse*; [b] = p< 0.05 vs SNP-1 mg treated diabetic rats; [d] = p<0.05 vs SNP-1 mg treated & SNP 1mg + EHNA treated diabetic rats; [e] = p<0.05 vs SNP-2 mg treated & SNP 2mg + EHNA treated diabetic rats.

NO deficiency resulted in renal stress and VED in hyperglycemic rats, while SNP prevented renal injury suggesting its additional role in our study for its nephroprotective action. The biochemical results obtained from the study further confirmed our hypothesis. Along with improving renal function, SNP prevented diabetes-induced renal hypertrophy and glomerulosclerosis.

CONCLUSION

On the basis of afore discussion, it may be concluded that in diabetes eNOS activity is down regulated resulting in reduced NO level. The purpose of the present study was to investigate the role of NO donor in the diabetic rats. Treatment with SNP alone or in the presence of PDE-2 inhibitor prevented renal dysfunction in dose dependent manner in diabetic rats. Moreover, administration of aldosterone in combination with SNP significantly attenuated SNP induced protective action. This confirmed that SNP showed protective action independent to PDE-2 activation. Nephroprotective effect of SNP is due to its antihypertensive, and antioxidant effect.

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