Research article

Evaluation of Protective Effect of Hydrogen Sulphide (H_2S) on Chemically-Induced Nephrotoxicity on Rats

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Abstract

Objective: To evaluate protective role of hydrogen sulphide (H_2S) donor on nephrotoxicity induced by gentamycin or diabetes in rats. **Methods:** Fifty adult male albino rats weighing ٢٥٠-٣٥٠ g were randomly divided into ٥ groups, ten rats in each group, as follows: (١) control group, (٢) streptozotocin group received single dose of streptozotocin ٥٠ mg/kg i.p, (٣) Streptozotocin + H_2S group received single dose of ٦٠ µmol/kg NaHS i.p. ٤٥ min before streptozotocin,(٤) Gentamicin group received single dose of gentamicin ٤٠ mg/kg i.p, and (٥) Gentamicin+H_2S group: received single dose of ٦٠ µmol/kg NaHS i.p. ٤٥ min before gentamicin. Rats were sacrificed after one week. Blood samples were collected and ١g of kidney tissue was homogenized. The assessed parameters were serum urea, creatinine, sodium, potassium and albumin. Urinary creatinine and renal tissue malondialdehyde (MDA) and superoxide dismutase (SOD) activity were also assessed. **Results:** Pretreatment of both diabetic and gentamicin nephrotoxic groups by H_2S donor significantly reduced levels of serum urea, serum creatinine, urinary creatinine, serum sodium and serum potassium, with increased level of serum albumin in comparison to the corresponding nephrotoxic groups. Pretreatment with H_2S donor significantly decreased renal MDA level in both diabetic and gentamicin nephrotoxic groups and significantly increased SOD activity in diabetic nephrotoxic group in comparison to the non-treated corresponding nephrotoxic group. **Conclusion:** Hydrogen sulphid has a renal protective effect against diabetic nephropathy and gentamicin induced nephrotoxicity that is initiated through its anti-oxidant properties.

Keywords: Hydrogen sulphide; gentamicin; diabetes; nephrotoxicity

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Introduction

Nephropathy is a leading cause of morbidity and mortality and its prevalence is continuously increasing[١]. Diabetic nephropathy (DN) is the most common cause of the chronic kidney disease in the world[٢]. DN greatly increases the risk of premature death by end stage renal disease and is associated with increased cardiovascular mortality. Therefore, huge research efforts are focused on deciphering pathologic molecular mechanisms in DN, which may provide valuable tools for early diagnosis and prevention of DN onset and evolution[٣].

Hydrogen sulphide (H_2S), which is recognized as the third gasotransmitter, identified after nitric oxide (NO) and carbon monoxide, is endogenously generated by cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), and ٣-mercaptopyruvate sulfurtransferase (٣MST). In recent years, accumulating evidence has demonstrated that H_2S plays critical roles in the pathophysiology of chronic kidney diseases [٤]. Therefore, the aim of this work is to investigate the beneficial effects of H_2S donor in both gentamicin and diabetic nephropathy.

Materials and Methods

The present study was conducted on adult male albino rats weighing ٢٥٠-٣٥٠ g. Rats were fed a standard diet of commercial rat chow and tap water and left to acclimatize to the environment for at least one week prior to inclusion in the experiments.

Adult male albino are divided into the following groups each of ten rats: control group and nephrotoxic groups including a) Streptozotocin group: received single dose of streptozotocin ٥٠ mg/kg i.p, b) Streptozotocin +H٢S group: received single dose of ٦٠ µmol/kg NaHS i.p. ٤٥ min before streptozotocin, c) Gentamicin group: received single dose of gentamicin ٤٠ mg/kg i.p, and d) Gentamicin+H٢S group: received single dose of ٦٠ µmol/kg NaHS i.p. ٤٥ min before gentamicin.

After one week the animals were sacrificed. Blood samples were collected by decapitation and centrifuged at ٥٠٠٠ R.P.M. for ٥ minutes for serum collection using centrifuge. Sera were kept at -٨٠°C until assessment of various parameters. ١g of kidney tissue was homogenized in ١٠ ml of phosphate buffer saline (PBS) and kept at -٨٠°C until assessment of various parameters. Serum parameters; urea, createnine, sodium, potassium and albumin were assessed. Urinary creatinine and renal tissue MDA and SOD activity were also assessed.

Serum urea was assayed using an enzymatic colorimetric kit based on modified Borthelot reaction[٥]. Serum and urinary creatinine was determined using a kinetic colorimetric creatinine kit based on Jaffe reaction[٦]. Serum contents of Na+ and potassium K+ were determined as previously described[٧]. Modified bromocresol green colorimetric method was used to determine serum albumin. The renal contents of lipid peroxides were assayed by a spectrophotometric method based on the reaction between MDA and thiobarbituric acid[٨]. SOD activity was determined according to previously described method[٩], based on the fact that the autooxidation of pyrogallol is inhibited by SOD.

Statistical analysis was performed using Graph Pad Prism, version ٥.٠١ for Windows (Graphpad Software, San Diego California USA). Data were expressed as mean ± standard error of the mean (S.E.M.). One-way analysis of variance (ANOVA) followed by Tukey-post analysis test were used to analyze the results for statistically significant difference. P value less than ٠.٠٥ were considered significant.

RESULTS

The results of the present study indicated that injection of either streptozotocine or gentamicin was associated with a significant elevation in the serum urea and creatinine levels in comparison to control group. Also, administration of NaHS into diabetic and gentamicin nephrotoxic groups led to significant reduction in the serum urea and creatinine levels in comparison to the corresponding nephrotoxic groups (Table ١).

Injection of either streptozotocine or gentamicin was associated with a significant elevation in the urinary creatinine level in comparison to the control group. Pretreatment of both diabetic and gentamicin nephrotoxic groups by NaHS significantly reduced urinary creatinine level in comparison to the corresponding nephrotoxic groups (Figure ١).

Administration of streptozotocine or gentamicin led to significant elevation of the serum sodium and potassium levels in comparison to the control group. However, pretreatment by H٢S donor led to significant reduction of the serum sodium and potassium levels in both diabetic and gentamicin groups in comparison to the corresponding nephrotoxic groups(Table ٢).

Serum albumin level significantly decreased after administration of streptozotocine and gentamicin in comparison to control group, and it significantly increased after H٢S donor pretreatment of both diabetic and gentamicin nephrotoxic groups in comparison to the corresponding nephrotoxic group (Figure ٢).

Strptozotocine induced diabetes and gentamicin injection led to significant increase in the renal tissues level of MDA compared to control group. However, injection of H٢S donor significantly decreased the renal MDA level in both diabetic and gentamicin nephrotoxic groups in comparison to the non-treated corresponding nephrotoxic group (Figure ٣).

Injection of streptozotocine but not gentamicin led to significant decrease in the renal SOD activity in comparison to the control group. Pretreatment with H٢S donor significantly increased the SOD activity in the diabetic nephrotoxic group in comparison to the corresponding non pretreated group (Figure ٤).

Table ١: Effect of NaHS (٦٠µml/kg) on serum urea and creatinine of chemically induced nephrotoxic rats

Group	Serum urea (mg/dL)	Serum creatinine (mg/dL)
Control	٤٨.٤١ ± ١.٧٦ ٤٠.٢٤-٥٨.٥٤	١.٦١٥±٠.٠٩٢ ١.٢٣١-٢.١٥٤
Diabetic	٢٩٧.٥ ± ٩.٧٣ a ٢٥٦.٧-٣٦٤.٦	٦.٣٧٢±٠.٣٦٨ a ٥.٠٧٧-٩.٨٤٦
Diabetic+ H٢S	٩٨.٨٧ ± ٣.٧٥ a b ٧٥.٦١-١١٨.٣	٣.٦٥٤±٠.١٢٤ a b ٣.٠٧٧-٤.٧٦٩
Gentamicin	١١٨.٣ ± ٢.٣١ a ١٠٧.٩-١٣٢.٣	٣.٩٦٤±٠.٣٣١ a ٣.٠٧٧-٧.٦٩٢
Gentamicin+H٢S	٧٤.٣٤ ± ١.٨٧ a c ٦٢.٨٠-٨٣.٥٤	٣.٦٦٩±٠.١٤٦ a ٢.٣٠٨-٤.٣٠٨

Values represent the mean ± SEM and range. Groups are compared by Tukey-post test. [a] significance from control group at p < ٠.٠٥, [b] significance from diabetic group at p < ٠.٠٥, [c] significance from gentamicin group at p < ٠.٠٥.

Table ٢: Effect of NaHS (٦٠µml/kg) on serum sodium and potassium of chemically induced nephrotoxic rats

Group	Serum sodium (mmol/L)	Serum potassium (mmol/L)
Control	٤.٦٠ ± ٠.١٦ ٤-٥	١٠.٣٠ ± ٠.٢١٣٤ ٩-١١
Diabetic	٧.٧٥ ± ٠.١٨ a ٧-٩	١٤.٨٣ ± ٠.٢٠٧٢ a ١٤-١٦
Diabetic+ H٢S	٥.٠٨ ± ٠.٢١ b ٤-٧	٨.٠٠٠ ± ٠.١٦٠١ a b ٧-٩
Gentamicin	٦.٥٤ ± ٠.١٤ a ٦-٧	١٤.٩٢ ± ٠.٢١٠٧ a ١٤-١٦
Gentamicin+H٢S	٥.٠٨ ± ٠.١٤ c ٤-٦	٧.٧٦٩ ± ٠.٢٨٠٩ a c ٦-٩

Values represent the mean ± SEM and range. Groups are compared by Tukey-post test. [a] significance from control group at p < ٠.٠٥, [b] significance from diabetic group at p < ٠.٠٥, [c] significance from gentamicin group at p < ٠.٠٥.

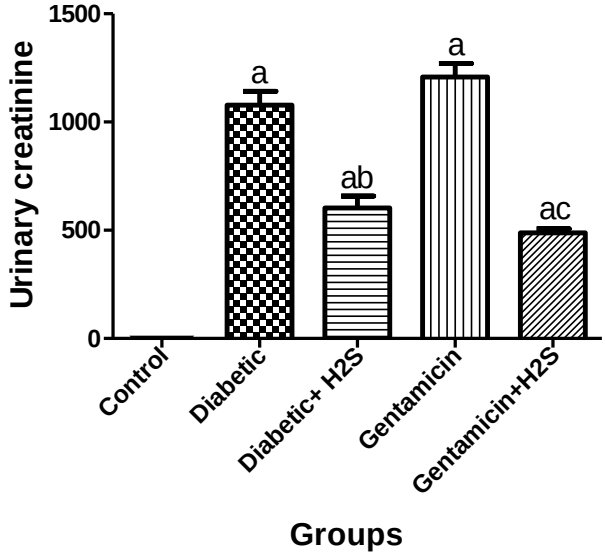

Fig. ١: Effect of NaHS (٦٠µml/kg) on urinary creatinine of chemically induced nephrotoxic rats. Values represent the mean ± SEM. Groups are compared by Tukey-post test. [a] significance from control group at $p < ٠.٠٥$, [b] significance from diabetic group at $p < ٠.٠٥$, [c] significance from gentamicin group at $p < ٠.٠٥$.

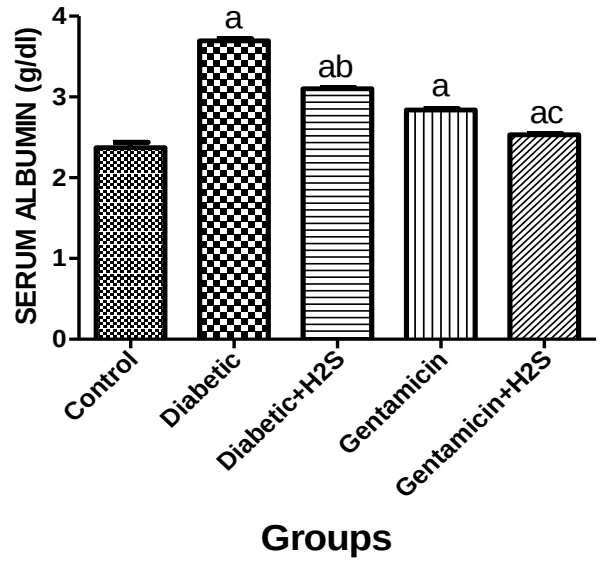

Fig. ٢: Effect of NaHS (٦٠µml/kg) on serum albumin of chemically induced nephrotoxic rats. Values represent the mean ± SEM (n=١٠-١٣). Groups are compared by Tukey-post test. [a] significance from control group at $p < ٠.٠٥$, [b] significance from diabetic group at $p < ٠.٠٥$, [c] significance from gentamicin group at $p < ٠.٠٥$.

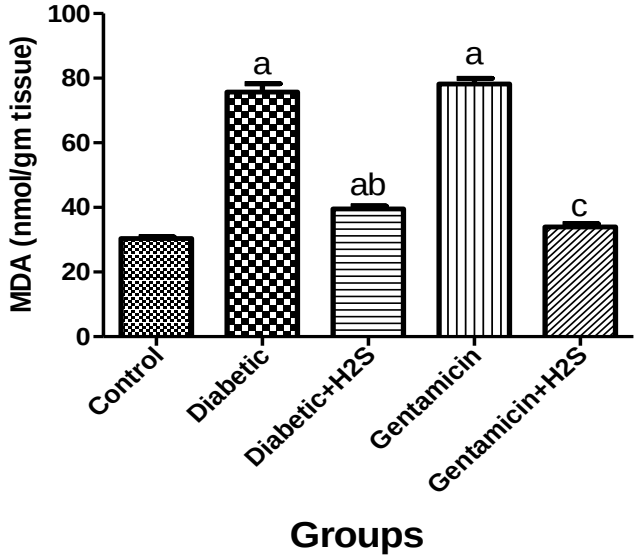

Fig. ٣: Effect of NaHS (٦٠µml/kg) on renal malondialdehyde (MDA) of chemically induced nephrotoxic rats. Values represent the mean ± SEM (n=١٠-١٣). Groups are compared by Tukey-post test. [a] significance from control group at $p < ٠.٠٥$, [b] significance from diabetic group at $p < ٠.٠٥$, [c] significance from gentamicin group at $p < ٠.٠٥$.

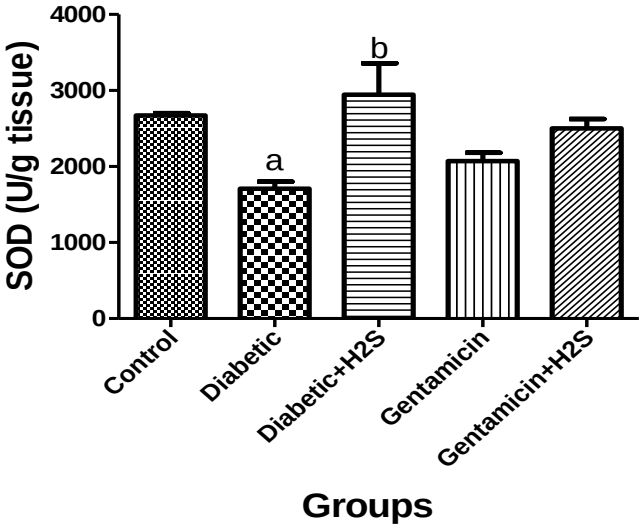

Fig. ٤: Effect of NaHS (٦٠µml/kg) on renal superoxide dismutase (SOD) of chemically induced nephrotoxic rats. Values represent the mean ± SEM (n=١٠-١٣). Groups are compared by Tukey-post test. [a] significance from control group at $p < ٠.٠٥$, [b] significance from diabetic group at $p < ٠.٠٥$.

Discussion

Nephropathy is defined as partial loss of function of kidney associated with nephritic syndrome, glomerulosclerosis and persistent albuminuria, declining GFR, elevated arterial blood pressure and fluid retention [١٠]. Possible causes of nephropathy may be due to administration of analgesics, aminoglycosides, MTX, long-term exposure to lead or its salts, cadmium and xanthine oxidase deficiency. Chronic conditions that can produce nephropathy include systemic lupus erythematosus, hypertension as well as diabetes mellitus (DM), which lead to diabetic nephropathy (DN) which refers to any deleterious effect

on kidney structure and/or function caused by DM[١١].

In this study, we established a streptozotocin-induced diabetic rat model to investigate the protective effects of H٢S against diabetic nephropathy. Our findings indicated that diabetes was accompanied by impaired renal function in the form of significant increase in both blood urea and creatinine which was antagonized by NaHS pretreatment. This result is in accordance with others[٤,١٢,١٣]. Xue et al.,[١٤] also reported that supplementation of H٢S attenuated hyperglycemia-induced elevations in ROS and renin-angiotensin system (RAS) activation. It was also reported that H٢S has therapeutic potential to prevent adverse diabetic renal remodeling[١٥]. Yamamoto et al.,[١٦] also reported that progressive diabetic nephropathy showed vasoconstriction and a loss of blood flow in renal peritubular capillary that was ameliorated by NaHS treatment.

The present results revealed that DN was associated with a significant increase in both serum Na+ and K+ levels as compared with control group. These results may explain by the fact that induction of DN lead to micro-angiopathesis and subsequent decrease in renal blood flow and GFR with alteration in the electrolytes levels. This result is in accordance to Al-Malki and El Rabey[١٧]. On the other hand the NaHS pretreatment produced a significant decrease in bothserum Na+ and K+ levels as compared with diabetic group which may be secondary to diuretic actions of NaHS is in accordance to other investigators[١٨].

Serum albumin is known as a predominant antioxidant in plasma[١٩]. More than ٧٠٪ of the free radical-trapping activity of serum is due to serum albumin[٢٠]. Decreased level of serum albumin *per se* is another complication of diabetes, because albumin plays a decisive role in modulating osmotic pressure of plasma. One possible explanation for this hypoalbuminemia may be the increased urinary excretion of albumin as a result of diabetic nephropathy [٢١]. In the present study diabetic rats have low serum albumin in comparison to normal control rats which is in accordance with other investigators [٢٢], which was corrected by pretreatment with NaHS which may be secondary to decrease in albuminuria as a result of protection from diabetic nephropathy as previously reported[٤,١٢,١٣].

It is well documented that hyperglycemia is associated with excessive free radical generation and oxidant stress and poor antioxidant status[٢٣]. Oxidative stress may damage cellular structures via lipid peroxidation of cellular membranes. Superoxide radical reacts with lipid to form lipid peroxides followed by β-oxidation to form MDA[٢٤]. When oxidation capacity overcomes the rate of antioxidants, MDA level is increased[٢٥]. Data of the present study provide a direct evidence for the peroxidation power of STZ. This was deduced by the significant rise in the renal level of MDA in DN rats compared to normal animals. These results are in accordance with those of Abo-Salem et al., [٢٦] who observed that oxidative damage is one of the pathogenic mechanisms that contribute to the development of DN.

Superoxide dismutase (SOD) is extensively distributed in all cells and has a significant shielding role against oxidative injury induced by ROS. In the present study renal SOD is decreased in diabetic rats, which is in accordance with Zhou et al.,,[٤]. The decrease in the SOD activity may be related to the increase in the intracellular levels of H٢O٢. Exogenous H٢S administration was accompanied by significant decrease in MDA level and increase in SOD activity which confirm the antioxidant property of H٢S.

Gentamicin is known to be highly nephrotoxic antibiotic like other aminoglycosides, causes nephrotoxicity by inhibiting protein synthesis inrenal cells. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting inacute tubular necrosis which can lead to acute renal failure[٢٧]. Data of the present study show that both serum creatinine and urea levels were increased significantly in the gentamicin-treated group when compared with control group.

These results confirm that kidney is very sensitive to gentamicin toxicity. Administration of NaHS afer gentamicin induced nephrotoxicity significantly lower serum urea and creatinine levels which are in accordance with some investigators[٢٨]. However, others[٢٩] showed controversial results that may be dose, race or disease related.

In the present study injection of gentamicin was associated with disturbed electrolyte balance in the form of excess Na+ and K+ level, which is in accordance with other investigators[٣٠], however the differences between experimental animals or duration might have led to the different results in other studies[٣١].

In the present study gentamicin injection was associated with hypoalbuminemia which may be secondary to increase in urinary albumin excretion[٣٢]. Pretreatment by NaHS abolished completely the hypoalbumiemic effect of gentamicin which confirms the renal protective effect of H٢S in gentamicin induced toxicity that was previously reported[٢٨].

In present study, gentamicin-induced nephrotoxicity was associated with increase in renal MDA level as a result of increase in free radical generation [٣٣] and decrease in SOD activity due to increased production of ROS and subsequent inactivation of antioxidant enzymes [٣٤]. The group that was given gentamicin and NaHS had significantly lower MDA levels and higher SOD activity in kidney tissue than those that was given gentamicin alone. This result is in agreement with Otunctemur et al., [٢٨] and confirms the antioxidant property of H٢S which was previously reported [٣٥-٣٧].

In conclusion, hydrogen sulphid has a protective effect against diabetic nephropathy and gentamicin induced nephrotoxicity in rats mainly by its antioxidant propriety.

ABBREVIATIONS

٣MST	٣-mercaptopyruvate sulfurtransferase
ANOVA	One-way analysis of variance
CBS	Cystathionine β-synthase
CSE	Cystathionine γ-lyase
DM	Diabetes mellitus
DN	Diabetic nephropathy
GFR	Glomerular Filtration Rate
H٢O٢	Hydrogen peroxide
H٢S	Hydrogen sulphide
MDA	Malondialdehyde
MTX	Methotrexate
NaHS	Sodium Hydrosulfide
NO	Nitric oxide
PBS	phosphate buffer saline
RAS	Renin-angiotensin system
ROS	Reactive Oxygen Species
SEM	Standard error of the mean
SOD	Superoxide dismutase
STZ	Streptozitocin

References

١. Ficociello LH, Perkins BA, Roshan B, Weinberg JM, Aschengrau A, Warram JH, Krolewski AS. Renal hyper-filtration and the development of microalbuminuria in type ١ diabetes. Diabetes Care. ٢٠٠٩ ;٣٢(٥):٨٨٩-٩٣.

٢. Ranjbar A, Ghasemi H, Hatami M, Dadras F, Heidary Shayesteh T, Khoshjou F. Temporal effects on diabetic nephropathy in male rats. J Renal Inj Prev. ٢٠١٦;٥(٢):٧٤-٨.

٣. Manda G, Checherita AI, Comanescu MV, Hinescu ME. Redox Signaling in Diabetic Nephropathy: Hypertrophy versus Death Choices in Mesangial Cells and Podocytes. Mediators Inflamm. ٢٠١٥; ٢٠١٥: ٦٠٤٢٠٨.

٤. Zhou X, Feng Y, Zhan Z٣, Chen J. Hydrogen sulfide alleviates diabetic nephropathy in a streptozotocin-induced diabetic rat model. J Biol Chem. ٢٠١٤; ٢٨٩(٤٢):٢٨٨٢٧-٣٤.

٥. Patton CJ, Crouch SR. Enzymatic method for determination of urea (urease-modified Bethelot reaction. Anal Chem. ١٩٧٧;٤٩:٤٦٤–٤٦٩.

٦. Schirmeister J., Willmann H., Kiefer H, HallauerW. Forand against the usefulness of endogenous creatinine clearance infunctional kidney diagnosis. Dtschmed Wochenschr. ١٩٦٤; ٨٩:١٦٤٠-٤.

٧. Hald PM. The flame photometer for the measurement of sodium and potassium in biological materials. J Biol Chem. ١٩٤٦;١٦٧:٤٩٩–٥١٠.

٨. Mihara M, Uchiyama M. Properties of thiobarbituric acid-reactive materials obtained from lipid peroxide and tissue homogenate. Chem.Pharm.Bull. ١٩٨٣; ٣١ (٢):٦٠٥-٦١١.

٩. Marklund S and Marklund G. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Er J. Biochem.١٩٧٤; ٤٧: ٤٦٩-٤٧٤.

١٠. Blickle JF, Doucet J, Krummel T, Hannedouche T. Diabetic nephropathy in the elderly. Diabetes Metab.٢٠٠٧;٣٣ Suppl ١:S٤٠-٥٥.

١١. Magee GM, Bilous RW, Cardwell CR, Hunter SJ, Kee F, Fogarty DG. Is hyperfiltration associated with the future risk of developing iabetic nephropathy? A meta-analysis. Diabetologia. ٢٠٠٩; ٥٢(٤):٦٩١-٧.

١٢. Safar MM, Abdelsalam RM. H٢S donors attenuate diabetic nephropathy in rats: Modulation of oxidant status and polyol pathway. Pharmacol Rep.٢٠١٥;٦٧(١):١٧-٢٣.

١٣. Qian X, Li X, Ma F, Luo S, Ge R, Zhu Y. Novel hydrogen sulfide-releasing compound, S-propargyl-cysteine, prevents STZ-induced diabetic nephropathy.Biochem Biophys Res Commun. ٢٠١٦. pii: S٠٠٠٦-٢٩١X(١٦)٣٠٤٧٥-٢.

١٤. Xue H١, Yuan P, Ni J, Li C,Shao D, Liu J, Shen Y, Wang Z, Zhou L, Zhang W, Huang Y, Yu C, Wang R, Lu L. H(٢)S inhibits hyperglycemia-induced intrarenal renin-angiotensin system activation via attenuation of reactive oxygen species generation.PLoS One. ٢٠١٣;٨(٩):e٧٤٣٦٦.

١٥. Kundu S, Pushpakumar SB, Tyagi A, Coley D, Sen U. Hydrogen sulfide deficiency and diabetic renal remodeling: role of matrix metalloproteinase-٩.Am J Physiol Endocrinol Metab. ٢٠١٣;٣٠٤(١٢):E١٣٦٥-٧٨.

١٦. Yamamoto J١, Sato W, Kosugi T, Yamamoto T, Kimura T, Taniguchi S, Kojima H, Maruyama S, Imai E, Matsuo S, Yuzawa Y, Niki I. Distribution of hydrogen sulfide (H٢S)-producing enzymes and the roles of the H٢S donor sodium hydrosulfide in diabetic nephropathy. Clin Exp Nephrol. ٢٠١٣;١٧(١):٣٢-٤٠.

١٧. Al-Malki AL and El Rabey HA. The antidiabetic effect of low doses of Moringa oleifera Lam. seeds on streptozotocin induced diabetes and diabetic nephropathy in male rats. Biomed Res Int. ٢٠١٥;٢٠١٥:٣٨١٠٤٠.

١٨. Ahmad FU, Sattar MA, Rathore HA, Tan YC, Akhtar S, Jin OH, Pei YP, Abdullah NA, Johns EJ. Hydrogen sulphide and tempol treatments improve the blood pressure and renal excretory responses in spontaneously hypertensive rats. Ren Fail. ٢٠١٤; ٣٦ (٤):٥٩٨-٦٠٥.

١٩. Roche M, Rondeau P, Singh NR, Tarnus E, Bourdon E. The antioxidant properties of serum albumin. FEBS Lett. ٢٠٠٨;٥٨٢(١٣):١٧٨٣-٧.

٢٠. Bourdon E١, Blache D. The importance of proteins in defense against oxidation. Antioxid Redox Signal. ٢٠٠١;٣(٢):٢٩٣-٣١١.

٢١. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type ٢ diabetes: progressive, interrelated, and

independently associated with risk of death. Diabetes. ٢٠٠٢;٥١(٤):١١٥٧-٦٥.
٢٢. Park KT, Yun CH, Bae CS, Ahn T. Decreased level of albumin in peripheral blood mononuclear cells of streptozotocin-induced diabetic rats. J Vet Med Sci. ٢٠١٤;٧٦(٨):١٠٨٧-٩٢.
٢٣. Piwkowska A, Rogacka D, Audzeyenka I, Jankowski M, Angielski S. High glucose concentration affects the oxidant-antioxidant balance in cultured mouse podocytes. J Cell Biochem. ٢٠١١;١١٢(٦):١٦٦١-٧٢.
٢٤. Injac R, Boskovic M, Perse M, Koprivec-Furlan E, Cerar A, Djordjevic A, Strukelj B. Acute doxorubicin nephrotoxicity in rats with malignant neoplasm can be successfully treated with fullerenol C٦(OH)٢٤ via suppression of oxidative stress. Pharmacological Reports. ٢٠٠٨; ٦٠(٥):٧٤٢-٩.
٢٥. Al-Saedi HF, Al-Zubaidy AA, Khattab YI. The Possible Effects of Montelukast against Doxorubicin-Induced Nephrotoxicity in Rabbits. International Journal of Advanced Research. ٢٠١٤; ٢(١١): ٧٢٣-٧٢٩.
٢٦. Abo-Salem OM, El-Edel RH, Harisa GE, El-Halawany N, Ghonaim MM. Experimental diabetic nephropathy can be prevented by propolis: Effect on metabolic disturbances and renal oxidative parameters. Pak J Pharm Sci. ٢٠٠٩;٢٢(٢):٢٠٥-١٠.
٢٧. Sundin DP, Sandoval R, Molitoris BA. Gentamicin inhibits renal protein and phospholipid metabolism in rats: implications involving intracellular trafficking. J Am Soc Nephrol. ٢٠٠١; ١٢(١):١١٤-٢٣.
٢٨. Otunctemur A, Ozbek E, Dursun M, Sahin S, Besiroglu H, Ozsoy OD, Cekmen M, Somay A, Ozbay N. Protective effect of hydrogen sulfide on gentamicin-induced renal injury. Ren Fail ٢٠١٤.;٣٦(٦):٩٢٥-٣١.
٢٩. Dam VP, Scott JL, Ross A, Kinobe RT. Inhibition of cystathionine gamma-lyase and the biosynthesis of endogenous hydrogen sulphide ameliorates gentamicin-induced nephrontoxicity. Eur J Pharmacol. ٢٠١٢;٦٨٥(١-٣):١٦٥-٧٣.
٣٠. Ezejiofor AN, Orish CN, Orisakwe OE. Costus afer ker gawl leaves against gentamicin-induced nephrontoxicity in rats. Iran J Kidney Dis. ٢٠١٤;٨(٤):٣١٠-٣.
٣١. Yazar E, Elmas M, Altunok V, Sivrikaya A, Oztekin E, and Birdane YO. Effects of aminoglycoside antibiotics on renal antioxidants, malondialdehyde levels, and some serum biochemical parameters Can J Vet Res. ٢٠٠٣; ٦٧(٣): ٢٣٩–٢٤٠.
٣٢. Patil CR, Jadhav RB, Singh PK, Mundada S, Patil PR. Protective effect of oleanolic acid on gentamicin induced nephrotoxicity in rats. Phytother Res. ٢٠١٠;٢٤(١):٣٣-٧.
٣٣. Kuhad A, Tirkey N, Pilkhwal S, Chopra K. Effect of Spirulina, a blue green algae, on gentamicininduced oxidative stress and renal dysfunction in rats. Fundam Clin Pharmacol. ٢٠٠٦;٢٠(٢):١٢١-٨.
٣٤. Karadeniz A, Yildirim A, Simsek N, Kalkan Y, Celebi F. Spirulina platensis protects against gentamicin-induced nephrotoxicity in rats. Phytother Res. ٢٠٠٨;٢٢(١١):١٥٠٦-١٠.
٣٥. Olson KR. A Practical Look at the Chemistry and Biology of Hydrogen Sulfide. Antioxidants and Redox Signaling. ٢٠١٢; ١٧:٣٢-٤٤.
٣٦. Jung KJ, Jang HS, Kim JI, Han SJ, Park JW, Park KM. Involvement of hydrogen sulfide and homocysteine transsulfuration pathway in the progression of kidney fibrosis after ureteral obstruction. Biochim Biophys Acta. ٢٠١٣;١٨٣٢(١٢):١٩٨٩-٩٧.
٣٧. Xie ZZ, Shi MM, Xie L, Wu ZY, Li G, Hua F, Bian JS. Sulfhydration of p٦٦Shc at cysteine٥٩ mediates the antioxidant effect of hydrogen sulfide. Antioxid Redox Signal. ٢٠١٤; ٢١(١٨): ٢٥٣١-٤٢.