Ameliorative Effects of Sodium Hydrogen Sulphide in the Renal Tissues of Diabetic Rats Orally Exposed to Monosodium Glutamate

LAMIDI W. B. OLANIYAN[1], BUSUYI D. KEHINDE[2], TAOFIK R. GBADAMOSI[3] ,JONATHAN A. ADEBISI[4]

[1,2]*Environmental and Drug Toxicology Research Unit, Biochemistry Department, Faculty of Pure and Applied Sciences, Ladoke Akintola University of Technology Ogbomoso, Nigeria*

[3]*Department of Research, Dave josh Global Research Laboratories Ogbomoso, Nigeria*

[4]*Department of Biochemical Science, Federal Polytechnic Ede, Nigeria*

Abstract- ***Diabetic mellitus is an autoimmune disease afflicting millions of population around the world. The concern is the increasing rate of the disease in spite of the advancement in medical education. Of the factors that predispose to the disease is the life style such as diet. Monosodium glutamate is a flavour enhancer widely believed to predispose to diabetes mellitus that is probably related to its obesogenic potential. However MSG direct involvement in the aetiology of diabetes mellitus remains controversial. The controversy is hinged on the finding that the portal blood levels of glutamate is lowly increased even after chronic ingestion since it is readily metabolized. This project therefore aimed to investigate the contribution of MSG to diabetes and possible amelioration by a sulphur compound specifically NaHS through attenuation of oxidative stress. Male Sprague Dawley rats were made diabetic by subcutaneous administration of alloxan and were fed MSG and or NaHS at 2 g/kg/day and 2.8 mg/kg/day respectively for 14 days. Non-diabetic rats were similarly treated or not treated. Rats renal post mitochondrial supernatants were analyzed for the specific activities of superoxide dismutase (SOD) and xanthine oxidase (XO) as well as reduced glutathione (GSH) concentration. N acetyl-β-D-glucosaminidase (NAG) specific activity and creatinine concentration in the urine and cystatin C (CysC) and urea in the serum were investigated. Histologic studies were carried out on the kidney tissues. Altered biochemical indices including induction of oxidative stress and pathological changes in cellular architecture were recorded among the diabetics, diabetics fed MSG as well as the non-diabetics fed MSG. Administration of NaHS to the rats attenuated the oxidative stress and ameliorated the pathological changes in the tissues. This work supports the theory that MSG induces renal dysfunction which is mediated by oxidative stress.***

Index Terms- ***Diabetes, monosodium glutamate, sodium hydrogen sulphide, renal toxicity.***

I. INTRODUCTION

Diabetes mellitus is a general term used for the body's failure to achieve euglycaemia and leading to hyperglyceamia. The disease is traditionally classified into type 1 diabetes in which the body fails to control blood sugar level following insulin insufficiency occasioned by the destruction of insulin secreting pancreatic β cells by the autoimmune system or the type 2 diabetes following cells' resistance to insulin. The concern about the disease is that the number of patients worldwide continues to rise and this is not unconnected with the nutritional status and the life style. Obesity is one of the predisposing factors to diabetes especially the Type 2 (WHO, 2018; Kahn et al., 2006). Monosodium glutamate (MSG), a food flavour enhancer, may play a role in the aetiology of diabetes mellitus following its obesogenic potential (Insawang et al., 2012; Roman-Ramos et al., 2011) and its compromised glucose utilization activity (Miskowiak et al., 1999) in experimental animals. However, demographic evidence linking diabetes or obesity in human to

MSG is still controversial. Under ordinary conditions, MSG is readily metabolized by humans, and portal blood level is thought to be lowly increased even after large dose (Walker and Lupien, 2000). Nephropathy is one of the microvascular complications of diabetes mellitus (Nazar, 2014). The kidney during diabetic complications undergoes structural changes such as thickening of glomerular basement membrane and nodule formation (Fowler, 2008). Oxidative stress has been reported as the underlying mechanism of MSG toxicities in the rat tissues (Abd Elkareem et al., 2021; Paul et al., 2012) as well as diabetes pathogenesis while reactive oxygen species are a critical factor in insulin secretion (Kajikawa et al., 2002). It is therefore thoughtful that an antioxidant molecule should play an ameliorative role in both MSG toxicity and diabetes. Hydrogen sulphide (H_2S) has been reported to play a number of biological functions (Sun et al., 2019) including involvement in diseases such as diabetes (Wang et al., 2020a; Qian et al., 2018). This investigation aimed to elucidate the biochemical and histological changes in the renal tissues of diabetic rats fed MSG and possible amelioration by exogenous hydrogen sulphide.

II. MATERIALS AND METHODS

All reagents were of analytical grade supplied from Sigma Aldrich Company, Louis USA and from RANDOX Laboratory Limited, UK. A brand of MSG manufactured by Ajinomoto Co., Inc. Tokyo, Japan was available commercially at a city grocery store.

Healthy male Wistar rats, body weight ranging from 185-200 g were obtained from the Institution's animal house. The rats were fed with animal feed pellets with access to drinkable water ad libitum. The animals were quarantined for 7 days and randomized by body weight into the test and the control groups (Table 1). There were 5 rats/group and were handled according to the Institutional guidelines on animal welfare. The test animals were exposed daily to MSG (2 g/kg body weight) and/or Sodium hydrogen sulphide (NaHS) (2.8mg/kg b w) in a total volume of 4 ml. normal saline solution via oral cannulation, for 14 days (Ronald and John, 2000; Rifat et al., 2018) Table 1.

Table 1. Animal grouping

Group	Dosing
Negative Control	Non diabetic rats + normal saline (N)
Positive Control	Diabetic rats + normal saline (D)
1	Diabetic rats + MSG (DM)
2	Diabetic rats + NaHS (DS)
3	Diabetic rats + MSG + NaHS (DMS)
4	Non diabetic + MSG (NM)
5	Non diabetic + NaHS (NS)
6	Non diabetic + MSG + NaHS (NMS)

Diabetes was induced in the rats by single subcutaneous injection of alloxan monohydrate (120 mg/kg b w) in 4 ml. normal saline (Mostafavinia et al., 2016) under anaesthesia consisting of 50 mg/kg ketamine hydrochloride administered intravenously along with 5 mg/kg diazepam. Onset of diabetes was confirmed after 72 hrs by the elevation of blood glucose (> 250 mg/dL), increased water consumption and frequent urination (Mostafavinia et al., 2016) which were sustained before the commencement of the dosing. The rats were sacrificed, and the kidneys harvested quickly and washed off blood and connective tissues with ice cold 1.15% KCl solution. The kidney was homogenized in ice-cold 0.25 M sucrose solution buffered with 40 mM Tris.HCl at pH 7.4 with a Potter Elvherjem Teflon-lined homogenizer. The homogenate was centrifuged at 8000g for 10 minutes in 20% (w/v) buffered medium. The pellet so obtained was taken up in 10ml. of the medium and re-centrifuged. The supernatants were centrifuged at 12000g for 10 minutes to remove light mitochondria. The final supernatants were combined and used as post-mitochondrial fraction for the biochemical analyses.

Biochemical Assays

In the urine samples, N-acetyl-β-D-glucosaminidase (NAG) specific activity was assayed by ELISA method (Kit No.CSB-E09450h .CUSABIO, China) following instructions as recommended by the manufacturer. Serum cystatin C concentration was determined by ELISA kit (Elabscience). Serum urea concentration was determined by colorimetric determination of ammonia using commercially

available Urease Berthelot's reagent kit MAK 120 (Sigma-Aldrich). Total protein concentration was determined using the method of Lowry et al. (1951). Urine creatinine concentration was determined spectrophotometrically according to Jaffe's reaction (Toora and Rajagopal, 2002). In the post-mitochondrial supernatants, superoxide dismutase (SOD) activity was determined spectrophotometrically by the inhibition of pyrogallol autoxidation (Marklund and Marklund, 1974). The sample was added after pyrogallol addition to the sample . The increased absorbance was measured at 420 nm. The activity was expressed as a function of protein to give specific activity of SOD. Reduced glutathione (GSH) concentration in the sample was determined by the method of Owens and Belcher (1965). Xanthine oxidase activity was determined according to the colorimetric method of Roussos (1967).

III. STATISTICAL ANALYSIS

Values are means ± standard error of mean. The difference between means was analyzed with ANOVA calculated by using GraphPad Prism software version 9. Tukey test was used for the Post hoc analysis. When the difference between means was substantial or statistically significant, $p < 0.05$. On the figure panes, the letter a represents the non-diabetic rats group (N), letter b represents the diabetic group (D), c for the diabetic fed NaHS (DS), d for the diabetics fed MSG (DM), e for the diabetics fed MSG and NaHS (DMS), f for the non-diabetics fed NaHS (NS), g for the non-diabetics fed MSG (NM), h for the non-diabetics fed MSG and NaHS (NMS).

IV. RESULTS

There was a significant increase in urinary specific activity of NAG among all forms of the diabetes-induced rats (D, DS, DM) save those administered MSG with NaHS (DMS) when compared with the non-diabetic control (N) (Fig. 1). The specific activity of NAG among DM group was significantly higher than the diabetic rats exposed to NaHS (DS). The DMS group had significantly low NAG activity when compared with D or DM. It was recorded that the non-diabetic rats exposed to MSG (NM) had significantly increased NAG specific activity when compared with group N (Fig. 1).

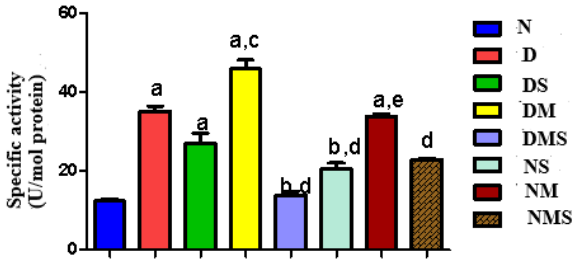

Figure 1. Urine N-acetyl-B-D-glucosaminidase specific activity

Cystatin C (CysC) concentration was assayed in the serum of the rats. Increase in the protein was recorded in somehow all the treated rats relative to the N and NMS groups (Fig. 2). The diabetic rats (D) had significantly high concentration of CysC when compared with the non-diabetic rats (N). The concentration was also high among non-diabetic rats exposed to MSG (NM) compared with the counterpart group that was additionally fed NaHS (NMS). But insignificantly low concentration was recorded in DS group when compared with group D.

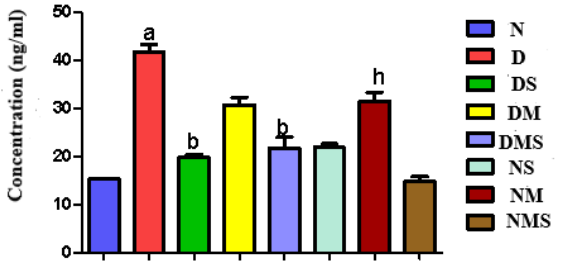

Figure 2. Serum cystatin C concentration.

Although urine creatinine concentration increased among rats in group D relative to group N, the difference was not substantial (Fig. 3) contrasting the results of NAG and cystatin C analyses. However, the increase in creatinine concentration among diabetic rats fed MSG (DM) was substantial when compared with N, D or DS group (Fig. 3). Among DMS, the creatinine concentration was reduced and was very substantial when compared with DS or DM. Very low creatinine concentration was recorded among rats in group DS. Also, low concentration was recorded among NS and NMS respectively and was very significant when compared with DM, DMS and NM. The increase in creatinine concentration among

rats in group NM was also substantial when compared with N, D, DS or DM. The low concentration recorded in group NMS was significant relative to DM or NM.

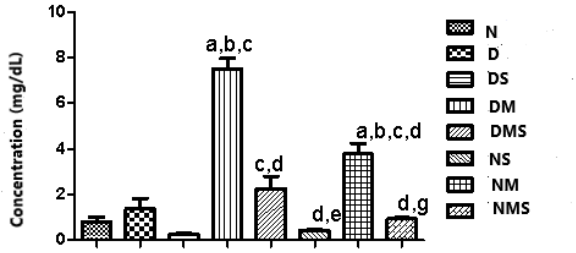

Figure 3. Urine creatinine

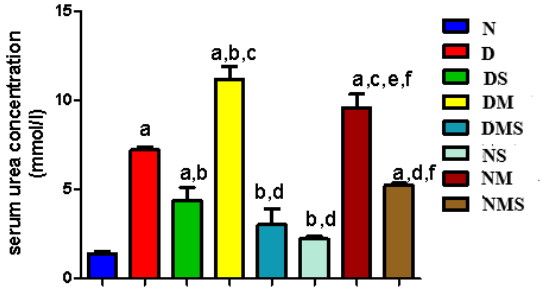

Figure 4. Serum urea concentration.

Increase in serum urea was recorded in all diabetic groups (D, DS, DM, DMS) as well as MSG treated group (NM) Fig. 4. Urea concentration substantially reduced among diabetic rats treated with NaHS (DS) compared with group D. Reduced urea was also observed among rats in group DMS which was significant when compared with DM. Among non-diabetics exposed to MSG (NM), increase in urea concentration significantly manifested when compared with the counterpart group that received additional NaHS (NMS).

Xanthine oxidase (XO) specific activity was higher among D, DS, DM and DMS than among N (Fig. 5). But the specific activity was significantly lower in DS than in D. Exposure of group D to MSG appear to significantly increase XO activity even when compared with DS. Similarly, exposure of non-diabetic group (N) to MSG (NM) significantly increased the activity when compared with N, DS or DMS. NaHS appear to be responsible for the significant reduction of the enzyme activity recorded among NMS, NS and DMS when compared with DM (Fig. 5).

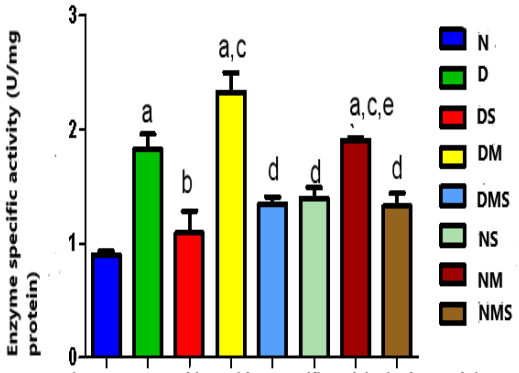

Figure 5. Rat xanthine oxidase specific activity in the renal tissue post-mitochondrial fraction.

The trend of specific activity of superoxide dismutase (SOD) among the experimental groups is as shown in Fig. 6. The enzyme activity was significantly increased in DS and DMS respectively. The increase may be associated with the NaHS which increased the enzyme activity in the non-diabetics (NS). The activity of SOD was reduced in the diabetics exposed to MSG (DM) Fig. 6.

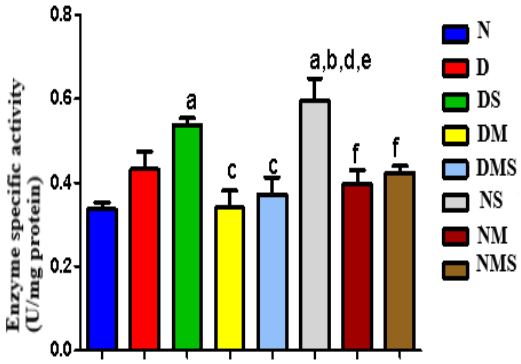

Figure 6. Superoxide dismutase specific activity in the rat renal tissue post-mitochondrial fraction.

A large increase in GSH concentration was recorded with the non-diabetics exposed to NaHS (NS) when compared with the non-diabetic control (N) (Fig 7). GSH concentration increased significantly in DS when compared with D. The difference of GSH concentrations between DMS and DS was significant which could be attributed to MSG exposure. In like manner, the high GSH concentration observed with the non-diabetic NMS compared with NM could be attributed to NaHS (Fig. 7).

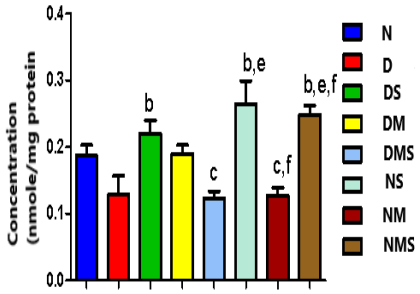

Figure 7. Reduced glutathione levels in the post-mitochondrial fraction of renal tissues of rats.

The results of the studies on histopathology of the renal tissues of the male rats are presented as Figures 8 to 15. The observed features and the pathological lesions are indicated with arrows in the micrographs. The control group presented kidneys with a normal cellular architecture (Fig. 8). In the D group (Fig. 9), rat kidney tissues showed poor cellular architecture. Sclerotic glomeruli among other lesions as shown in the micrograph (Fig. 9).

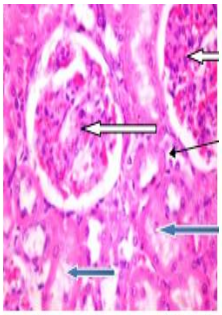

Figure 8. Photomicrograph of kidney sections of non-diabetic healthy rats stained by Haematoxylin and Eosin (H & E) showing normal architecture; the renal cortex shows normal glomeruli with normal mesengial cells and capsular spaces (white arrow), the renal tubules appear normal (blue arrow) and the interstitial spaces appear normal too (slender arrow).

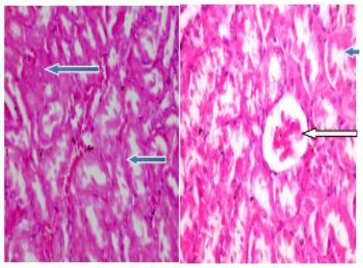

Figure 9. Photomicrographs of kidney sections of the diabetic rat stained H and E x400 showing poor architecture the renal cortex show glomeruli with sclerosis (white arrow), the renal tubules show moderate to severe tubular necrosis (blue arrow) and the presence of moderate vascular congestion.

Fewer of the pathological lesions recorded among the diabetic rats were seen when the rats were supplemented with NaHS (Fig. 10) just as the administration of NaHS to normal rats did not affect the kidney (Fig. 11).

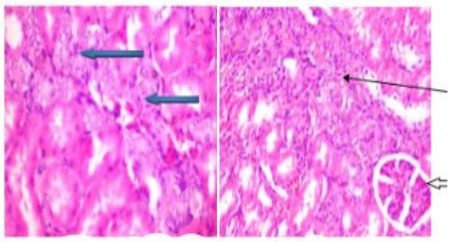

Figure 10. Photomicrographs of kidney sections of the diabetic rats fed NaHS 2.8 g/kg/day for 14 days showing poor cellular architecture; the renal cortex show normal glomeruli with normal mesengial cells and capsular spaces (white arrow), the renal tubules show degeneration of the epithelial cells (blue arrow), the interstitial show focal area of inflammatory cells (slender arrow) and mild vascular congestion seen. H & E x 400

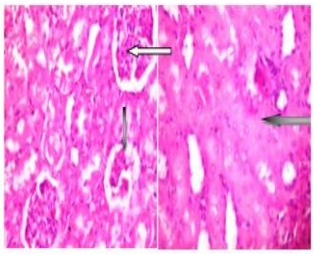

Figure 11. Photomicrographs (H & E stained) of kidney sections of normal healthy rats fed NaHS 2.8 mg/kg/day for 14 days showing moderately normal architecture; the renal cortex shows normal glomeruli with normal mesengial cells and capsular spaces (white arrow), few glomeruli with atrophy seen (black arrow), most of the renal tubules appear normal , some show necrotic degeneration (black arrow), the interstitial spaces appear normal (slender arrow). Mag x 400

MSG showed marked lesions whether applied to the healthy rats (Fig. 12) or to the diabetic rats (Fig. 13) which appeared lessened following respective administration of NaHS (Figs. 14 and 15).

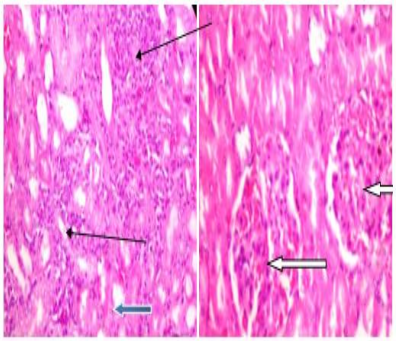

Figure 12. Photomicrographs (H & E) of kidney sections of normal healthy rats fed MSG 2 g/kg/day for 14 days showing poor architecture, the renal cortex show normal glomeruli with normal mesengial cells and capsular spaces (white arrow), the renal tubules appear normal (blue arrow), however, the interstitial spaces is severely infiltrated by inflammatory cells (slender arrow). Mag x 400

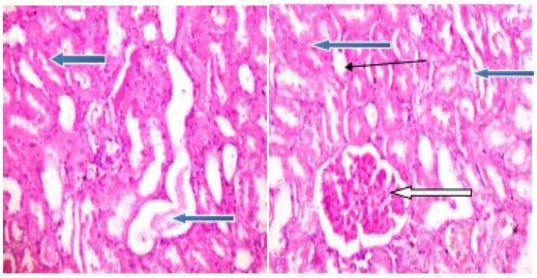

Figure 13. Photomicrographs (H & E) of kidney sections of diabetic rats fed MSG 2 g/kg/day for 14 days showing poor architecture; the renal cortex show normal glomeruli (white arrow), but the renal tubules show severe degeneration including tubular necrosis (blue arrow); the interstitial spaces appear normal (slender arrow). Mag x 400

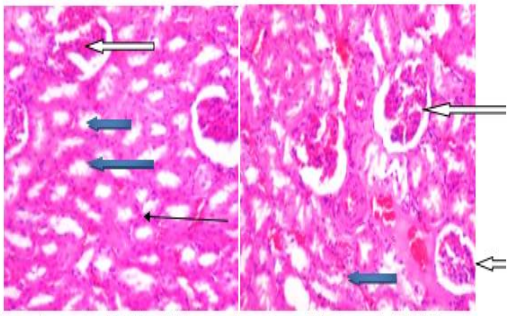

Figure 14. Photomicrographs (H & E) of kidney sections of non-diabetic rats fed MSG 2 g/kg/day+NaHS 2.8 mg/kg/day for 14 days showing normal architecture; the renal cortex show normal glomeruli with normal mesengial cells and capsular spaces (white arrow), the renal tubules appear normal (blue arrow), the interstitial spaces appear normal (slender arrow). Mag x 400

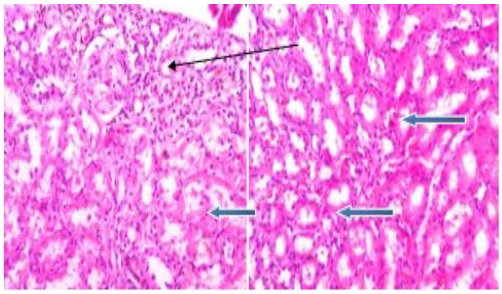

Figure 15. Photomicrographs (H & E) of kidney sections of diabetic rats fed MSG 2g/kg/day+NaHS 2 mg/kg/day for 14 days showing moderate architecture; the renal cortex show normal glomeruli (white arrow), but the renal tubules appear normal (blue arrow), the interstitial spaces show area of fibrosis (slender arrow). Mag x 400

V. DISCUSSION

Diabetic nephropathy is a chronic kidney disease in the diabetic patients. The disease is one of the most frequent microvascular complications of both types of diabetes mellitus (Nazar, 2014). N-acetyl-β-D-glucosaminidase (NAG) is a renal tubular lysosomal enzyme which activity has been shown to increase in patients with developing diabetic nephropathy (Driza et al., 2021). It is a marker of tubular cell dysfunction and a predictor of outcome in primary glomerulonephritis. Cystatin C (CysC), a low molecular weight (approximately 13.3 kilodaltons) protein, is removed from the bloodstream by glomerular filtration in the kidneys and and has been used as a biochemical marker for proximal tubular damage superior to serum creatinine (Sahoo et al., 2016; Bokenkamp et al., 2001). CysC has many properties that make it suitable as a marker of proximal tubular dysfunction (Séronie-Vivien et al., 2008). Such properties include its constant production and plasma concentration because it is encoded by a housekeeping gene (CST3) and no plasma protein binding. Endogenous creatinine clearance has been used as a measure of the glomerular filtration rate (GFR) and increased urine creatinine concentration suggests early sign of kidney dysfunction (Boudonck et al., 2009). Urea is the major end product of nitrogen metabolism in most animals and is produced in a series of reactions in the liver called the urea cycle. In the urea cycle, ammonia is converted to urea, which is carried by blood to the kidneys for elimination from the body. High levels of urea in the blood may indicate renal failure (Bamanikar et al., 2016). The results of NAG, CysC, creatinine and urea assays individually showed diabetes- and MSG-induced renal dysfunction in the rat and that the oral administration of NaHS which was the exogenous source of H_2S, reduced the severity of the renal impairment (Karmin and Siow, 2018) These outcomes appear to be supported by the results of the histopathology (Figures 8 - 15). H_2S has been reported as a regulator of mitochondrial ROS production (Fu et al., 2012). Oxidative stress, the imbalance between prooxidants and antioxidants favouring the former is the mechanism underlying the pathology of a number of diseases including diabetes (Pandarekandy et al., 2017). Xanthine oxidase (XO) catalyses the conversion of hypoxanthine/xanthine to uric acid and the reactive oxygen species which accumulation could lead to tissue damage. The increase in the specific activity of XO among the diabetic and MSG-fed rats showed that the the observed effects of these agents in the rats are mediated by reactive oxygen species (ROS) (Paul et al., 2012). Among the diabetic rats, ROS are produced by the persistent hyperglyceamia or by glycation (Bravi et al., 2006). SOD and GSH are antioxidants responsible for the

dismutation and neutralization of ROS. The increase in SOD activity by the non-diabetic rats NS could be attributed to NaHS administration. However, the observed increase among DS, DMS and NMS compared to DM could be a response to NaHS adminstration following oxidative challenge by diabetes and MSG (Paul, et al., 2012; Koya et al., 2003). The low GSH concentrations recorded among D, DM and NM, among DMS compared to DS as well as NM compared to NMS occasioned by diabetes or by MSG ingestion suggests onset of oxidative stress by GSH depletion (Maddaiah, 1990). The applied NaHS in NMS was obviously responsible for the increased GSH concentration which is a remedial effect. Both biochemical and histologic evidence showed that diabetes and or MSG caused renal dysfunction in the male rats and the NaHS supplementation reduced the severity of the renal dysfunction probably by the attenuation of the oxidative stress. Garlic and onion are good dietary sources of H_2S (Kim et al., 2018). Exogenous H_2S has been reported to ameliorate diabetic cardiomyopathy (Zhao et al., 2021) by inhibiting oxidative stress and apoptosis through activation of K_{ATP} channels, suppression of the NF-κB pathway and ROS production (Abd Elkareem et al., 2021; Tocmo and Parkin, 2019; Paul et al., 2012). It is believed that the kidney ranks after liver and gut as the leading producer of H_2S (Cao and Bian 2016). At the sub-cellular levels H_2S production is catalyzed by the so-called the three classic enzymes namely, 3-mercaptopyruvate thioltransferase, cystathionine-β-synthase and cystathionine-γ-lyase (Rose et al., 2017). Non-enzymatic pathways for endogenous H_2S generation have also been recognized (Yang et al., 2019). The endogenous sources of H_2S probably underscores the necessity of the molecule in cellular metabolism. NaHS, Na_2S and Sodium thiosulphate ($Na_2S_2O_3$) are presently identified as exogenous producers of H_2S. The first two are used in vitro and in animal models while thiosulphate is reported to be in use in medicine (Scammahorn et al., 2021). This work has provided additional data to the widely presumed contributory role of MSG to diabetic nephropathy mediated by ROS (Sharma, 2015; Pinterova et al., 2001). The overall evidence also showed that NaHS has promise in regulating renal insufficiency by attenuating oxidative stress.

CONCLUSION

MSG was toxic to the male rat kidney at the applied dosage and the toxicity was mediated by oxidative stress. NaHS is an antioxidant in the kidney and could be beneficial for the control of both MSG and diabetes-induced nephropathy.

REFERENCES

[1] Abd Elkareem M., Abd El Rahman M. A. M, Abou Khalil N S & Amer A S. Antioxidant and cytoprotective effects of Nigella sativa L. seeds on the testis of monosodium glutamate challenged rats. *Scientifc Reports* (2021) 11:13519 https://doi.org/10.1038/s41598-021-92977-

[2] Bamanikar S A, Bamanikar A A & Arora A. (2016). Study of Serum urea and Creatinine in Diabetic and nondiabetic patients in a tertiary teaching hospital. JMR 2(1): 12 - 15.

[3] Boudonck K J, Mitchell M. W., Nemet L., Keresztes L, Nyska A, Shinar D. & Rosenstock M. (2009). Discovery of Metabolomics Biomarkers for Early Detection of Nephrotoxicity. *Toxicologic Pathology* 37: 280-292

[4] Bravi MR, Armiento A, Laurenti O, Cassano-Faldetta M, De Luca O, Morettia A & De Mattia G.(2006). Insulin decreases intracellular oxidative stress in patient with type 2 diabetes mellitus. *Metab Clin Exp* 55: 591-696

[5] Cao X. & Bian J.S. (2016). The Role of Hydrogen Sulfide in Renal System. *Front. Pharm.* 7:385.

[6] Driza A R , Kapoula G V & Bagos P G (2021). Urinary N-Acetyl-β-D-glucosaminidase as an Indicative Biomarker of Early Diabetic Nephropathy in Patients with Diabetes Mellitus : A Systematic Review and Meta-Analysis. *Diabetology* 2: 272–285.

[7] Bokenkamp A., Ciarimboli G. & Dieterich C. (2001). Cystatin C in a rat model of end-stage renal failure. R*enal failure* 23(3&4): 431–438

[8] Brannan, T.S., Maker, H.O. & Raess, I.P. (1981). Regional distribution of catalase in adult rat brain. *J. Neurochem*. 86: 307- 309.

[9] Fowler M. J. (2008). Microvascular and macrovascular complications of diabetes. *Clin Diabetes* 26:77-82.

[10] Fu, M.; Zhang, W.; Wu, L.; Yang, G.; Li, H.& Wang, R. (2012). Hydrogen sulfide (H_2S) metabolism in mitochondria and its regulatory role in energy production. *Proc. Natl. Acad. Sci. USA* 109: 2943–2948.

[11] Hassan, A. Behjat, S. Mehri, K. Nima, S. Mohammed, E. Mina, R. & Parisa, A (2018). Protective effects of hydrogen sulfide on chronic kidney disease by reducing oxidative stress, inflammation and apoptosis. *EXCLI J.* 17: 14–23.

[12] Insawang T, Selmi C, Cha'on U, Pethlert S, Yongvanit P, Areejitranusorn P, Boonsiri P, Khampitak T, Tangrassameeprasert R, Pinitsoontorn C, Prasongwattana V, Gershwin ME & Hammock BD. (2012). Monosodium glutamate intake is associated with the prevalence of metabolic syndrome in a rural Thai population, *Nutr. Metab.* 9 (1); 50.

[13] Kahn S. E, Hull RL & Utzschneider KM. (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature Publishing Group*. 444(7121):840–846.

[14] Kajikawa M, Fujimoto S, Tsuura Y, Mukai E, Takeda T, Hamamoto Y, Takehiro M, Fujita J, Yamada Y, & Seino Y (2002). Ouabain Suppresses Glucose-Induced Mitochondrial ATP Production and Insulin Release by Generating Reactive Oxygen Species in Pancreatic Islets. *Diabetes* 51:2522–2529.

[15] Karmin O. & Siow Y. L. (2018). Metabolic imbalance of homocysteine and hydrogen sulfide in kidney disease. *Curr. Med. Chem.* 25: 367–377.

[16] Kim S, Kim DB, Jin W, Park J, Yoon W, Lee Y, Kim S, Lee S, Kim S, Lee OH, Shin D & Yoo M (2018). Comparative studies of bioactive organosulphur compounds and antioxidant activities in garlic (*Allium sativum L.*), elephant garlic (*Allium ampeloprasum L.*) and onion (*Allium cepa L.*). *Nat. Prod. Res*. 32:1193–1197.

[17] Koya D, Hayashi K, Kitada M, Kashiwagi A, Kikkawa R & Haneda M. (2003). Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats. *J Am Soc Nephrol* 14: S250-S253

[18] Jubaidi, F. F., Mathialagan, R. D., Noor, M. M., Taib, I. S. & Budin, S. B. (2019). Monosodium glutamate daily oral supplementation: Study of its effects on male reproductive system on rat model. *Syst. Biol. Reprod. Med.* 65, 194–204.

[19] Maddaiah V. T. (1990). Glutathione correlates with lipid peroxidation in liver mitochondria of triiodothyronine – injected hypophysectomised rats. *FASEB J*. 4:1513 –1518.

[20] Marklund S. & Marklund G. (1974). Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. *Eur. J. Biochem*. 47:469-474.

[21] Mostafavinia A., Amini A., Ghorishi S. K., Pouriran R. & Bayat M. (2016). The effects of dosage and the routes of administrations of streptozotocin and alloxan on induction rate of type 1 diabetes mellitus and mortality rate in rats. *Lab Anim Res* 32(3):160-165. http://dx.doi.org/10.5625/1ar.2016.32.3.160

[22] Miskowiak B., Kesa B., Limanowski A., Pantayka M., Filipiak B. & Folia M. (1999): Long-term effect of neonatal (MSG) treatment on reproductive system of the level rats. *Folia Morphol (Warsz)* 58(2): 105 –113

[23] Nazar & C. M. J. (2014). Diabetic Nephropathy; Principles of Diagnosis and Treatment of Diabetic Kidney Disease. *J. Nephropharmacol.* 3: 15–20

[24] Pinterova, L.; Zelezna, B.; Fickova, M.; Macho, L.; Krizanova, O.; Jezova, D. & Zorad S. (2001). Elevated AT1 Receptor Protein but Lower Angiotensin II-Binding in Adipose Tissue of Rats with Monosodium Glutamate-Induced Obesity. *Hormone and Metabolic Research* , 33(12), 708–712.

[25] Owens C. W. I. & Belcher R. V. (1965). A colorimetric micro-method for the determination of glutathione. *Biochem J.* 94(3): 705–711

[26] Pandarekandy S. T., Sreejesh P. G., Thampi B. S. H. & Sreekumaran E. (2017). Hypoglycaemic Effect of Glibenclamide: A Critical Study on the Basis of Creatinine and Lipid Peroxidation

Status of Streptozotocin-induced Diabetic Rat. *Indian J Pharm Sci* 79(5):768-777 [27] Park C. H., Choi S. H., Piao Y., Kim S., Lee, Y. J., Kim H. S., Jeong, S. J., Rah J. C., Seo, J. H., Jung J. & Suh, Y. H. (2000). Glutamate and aspartate impair memory retention and damage hypothalamic neurons in adult mice. *Toxicol. Lett*. 115(2): 117-125

[27] Paul M V, Abhilash M, Varghese M V, Alex M & Nair R H (2012). Protective effects of α-tocopherol against oxidative stress related to nephrotoxicity by monosodium glutamate in rats. *Toxicol Mech Methods* 22(8):625-630. doi: 10.3109/15376516.2012.714008.

[28] Praputpittaya, C. & Wililak, A. (2003): Visual performance in monosodium glutamate-treated rats. *Nutr. Neurosci*. 6(5): 301- 307.

[29] Qian, L.-L., Liu, X.-Y., Chai, Q., & Wang, R.-X. (2018). Hydrogen sulfide in diabetic complications: focus on molecular mechanisms. *Endocr. Metab. Immune Disord. Drug Targets* 18: 470–476. doi: 10.2174/1871530318666180426100532

[30] Rosalki S. B.& Tarlow D. (1974). Optimized Determination of γ-Glutamyltransferase by Reaction-Rate Analysis. *Clinical Chemistry* 20(9):1121–1124.

[31] Rose, P., Moore, P. K., & Zhu, Y. Z. (2017). H_2S biosynthesis and catabolism: new insights from molecular studies. *Cell. Mol. Life Sci.* 74: 1391–1412.

[32] Roussos G. G. (1967). Xanthine oxidase from bovine small intestine. In: Grossman L, Moldave K, (editors). New York (NY): Academic Press; pp. 5-16. *Methods in Enzymology* vol XII

[33] Sahoo P. R., Swain P., Nayak S. M., Kar D & Mishra S. R. (2016). Cystatin C: A new biochemical marker in livestock sector. *Journal of Advanced Veterinary and Animal Research*, 3(3): 200-205

[34] [35] Scammahorn J. J., Nguyen I T. N., Bos E. M. , Van Goor H. & Joles J. A. (2021). Fighting Oxidative Stress with Sulfur: Hydrogen Sulfide in the Renal and Cardiovascular Systems. *Antioxidants* 10: 373

[35] Sharma, A. (2015). Monosodium glutamate-induced oxidative kidney damage and possible mechanisms: a mini-review. *J Biomed Sci* 22: 93.

[36] Séronie-Vivien S, Delanaye P, Piéroni L, Mariat C, Froissart M & Cristol J P (2008). Cystatin C: current position and future prospects. *Clin Chem Lab Med.* 46(12):1664-1686.

[37] Sheu J. Y., Chen P. H., Tseng W. C., Chen C. Y. & Tsal L. Y. (2003). Spectrophotometric determination of a thiobarbituric-acid reactive substances in human hair. *Analytical Sciences* 19: 957 – 960.

[38] Sun H-J, Wu Z-Y, Cao L, Zhu M-Y, Liu T-T, Guo L, Lin Y, Nie X-W & Bian J-S. (2019). Hydrogen sulfide: recent progression and perspectives for the treatment of diabetic nephropathy. *Molecules* 24, 2857. doi: 10.3390/molecules24152857

[39] Tocmo, R., & Parkin, K. (2019). S-1-propenylmercaptocysteine protects murine hepatocytes against oxidative stress via persulfidation of Keap1 and activation of Nrf2. Free Radic. Biol. Med. 143, 164–175. doi: 10.1016/j.freeradbiomed.2019.07.022

[40] Toora B. D. & Rajagopal G. (2002). Measurement of creatinine by Jaffe's reaction--determination of concentration of sodium hydroxide required for maximum color development in standard, urine and protein free filtrate of serum. *Indian J Exp Biol*. 40(3):352-354

[41] Walker R. & Lupien J. R. (2000). The Safety Evaluation of Monosodium Glutamate. *The Journal of Nutrition,* 130(4), 1049S–1052S. https://doi.org/10.1093/jn/130.4.1049S

[42] Wang, H., Shi, X., Qiu, M., Lv, S., & Liu, H. (2020a). Hydrogen sulfifide plays an important protective role through influencing endoplasmic reticulum stress in diseases. *Int. J. Biol. Sci.* 16, 264–271. doi: 10.7150/ijbs.38143

[43] Wang H., Shi X., Qiu M., Lv S. & Liu H. (2020a). Hydrogen sulfide plays an important role by influencing NLRP3 inflammasome. *Int. J. Biol. Sci*. 16, 2752–2760. doi: 10.7150/ijbs.47595

[44] WHO (2018). World Health Organisation. https://www.who.int/news-room/fact sheets/detail/obesity-and-overweight, 2018

[45] Yang J., Minkler P., Grove D., Wang R., Willard B., Dweik R. & Hine C (2019). Non-enzymatic hydrogen sulfide production from cysteine in blood is catalyzed by iron and vitamin B-6. *Commun. Biol*. 2, 19. doi: 10.1038/s42003-019-0431-5

[46] Zanfirescu A, Ungurianu A., Tsatsakis A. M., Nițulescu G. M., Kouretas D., Veskoukis A., Tsoukalas D., Engin A. B., Aschner M. & Margină D. (2019). A review of the alleged health hazards of monosodium glutamate. *Compr. Rev. Food Sci. Food Saf.* 18, 1111–1134

[47] Zhao S, Li X, Li X, Wei X & Wang H. (2021). Hydrogen Sulfide Plays an Important Role in Diabetic Cardiomyopathy. *Front. Cell Dev. Biol*. 9:627336. doi: 10.3389/fcell.2021.627336