Effect of methionine – Schiff base on renal damage: Physiological and histological study

Dr. Jalal M. Mejbel
Al-Farahidi University, College of Medical Technology, Department of Anesthesia, Baghdad, Iraq
Corresponding author email: jalalafandimejbel@gmail.com

Dr. Fadhil H. Ahmed
Al-Farahidi University, College of Medical Technology, Department of Medical Lab Technology Baghdad, Iraq

Dr. Nour Taqi Khudair
Al-Farahidi University, College of Medical Technology, Department of Anesthesia, Baghdad, Iraq

Abstract---Methionine, an important amino acid, was employed to make the Schiff base used in this study. In damaged kidney tissue of rats, methionine-Schiff base functions as an antioxidant, anti-inflammatory, and neutralizer of free radicals such as sodium nitrite. Sodium nitrite, a common food preservative that has an important harmful influence on oxidative stress markers and renal tissue. It is generally known that nitrites can have harmful effects on mammals, including gonadotoxicity, hepatotoxicity, neurotoxicity, and carcinogenicity. According to antioxidant markers and microscopic investigation in this study, the groups treated with methionine-Schiff base had a better outcome than the group treated with sodium nitrite when compared to the control group.

Keywords---antioxidant, kidney injury, methionine, Schiff base, sodium nitrite.

Introduction

Schiff bases (Hugo Schiff) are substances having the general formula R1CR2=NR3, where R1, R2, and R3 are organic side chains. Because azomethine is present in the Schiff base formula, it is now R1CH=NR3 (R1 and R2 may be hydrogens, and R3 is a hydrogen). The carbonyl (C=O) and amino (R-NH2) derivatives of Schiff bases have been reported to play a key role in the development of numerous medications. Antibacterial, antifungal, antiviral,
antiparasitic, anticancer, anti-inflammatory, and antioxidant properties are among the many biological applications of Schiff bases (Zou et al. 2016; Islam et al. 2018; Noguchi and Niki 2019).

\[ \text{Figure 1: General structure of Schiff bases} \]

Methionine [2-amino-4- (methylthio) butanoic acid] is an important amino acid that is used in the biosynthesis of proteins. It contains a α-amino group, a α-carboxylic acid group, and an S-methylthio ether side chain, classifying it as a nonpolar, aliphatic amino acid (Anand and Sati 2013; Taş, Şenocak, and Aydin 2018). Antioxidants such as ascorbic acid, Tocopherols, glutathione, and selenium, which are defined as substances that can deactivate or reducing the risk of free radicals attacking live cells. In order to sustain good cellular and systemic health and wellbeing functions, they are essential. In the event of free radical exposure such as sodium nitrite (NaNO2) which contain nitric oxide (NO2), from a variety of sources, has lead organisms in help of antioxidant to develop a series of defense mechanisms involved in the prevention of cellular damage and a variety of diseases (Fouad et al. 2017; Widyastuti et al., 2019).

Food additives can be natural or synthetic, which are added to increase expire date and maintain quality of food by inhibiting or arrest its fermentation, acidification, decomposition, and contamination (Peng et al., 2018). Sodium nitrite (NaNO2) is a common preservative for manufactured food, especially meat product which prevents the growth of microorganism, preserve the color, and offer specific flavor. Through the oral route, human are frequently exposed to NaNO2 because they use it as a food additive.

Some vegetables, such as spinach and some well water, contain high concentrations of nitrates (Singh et al., 2014; Widyastuti 2013; Ansari et al. 2018). They used NaNO2 as free acid or salt when eaten will disband to nitroxide (NO) and oxide (O). Nitroxide (NO) binds to red blood cell to form nitroso-hemoglobin, which reducing the ability of erythrocyte to fixation of oxygen (Ateya et al. 2016). NaNO2 influences several organ functions, include kidney, liver, esophagus, stomach, pancreas, bladder, etc. The kidney has an important role to release the toxins. Nitrite ions from NaNO2 are soluble in water and may cause some effect for kidney during its tubular reabsorption. (Azzouz and Ali 2010; Singh., et al., 2014; Datta and Ramya 2012)

NaNO2 consumption increases the releasing activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes which cause the development of cytotoxic N-nitroso compound. These compounds trigger severe hepatic and renal necrosis and cause kidney disfunction. The kidney
tissue damage also can reveal by an examination of the creatinine and urea concentration in the serum. Kidney tissue damage is recognizable because of doses of 60-75 mg/kg body weight NaNO2 in the feeding of rat (Suvarna et al.,2018; Widyastuti 2013; Hasani and Najim 2017).

Malonaldehyde (MDA) is one of the lipid peroxidation products. Lipid peroxidation refers to the oxidative breakdown of lipids. (Niki, 2008). Superoxide dismutases (SODs) are a class of persistently associated enzymes that catalyze the analysis of the superoxide anion into oxygen and hydrogen peroxide ,Superoxide dismutase are found in nearly totally aerobic cells and in extracellular fluids (Johnson & Giulivi, 2005). Superoxide dismutases comprises metal ion cofactors like zinc, copper, iron or manganese. In human being, the extracellular and cytoplasmic SOD encompasses zinc and, copper whereas mitochondrial enzyme comprising manganese. Given that mice lacking this enzyme pass away shortly after giving birth, the mitochondrial SOD isozyme is the most physiologically relevant one. (Nozik-Grayck et al., 2005).

The enzymes catalase These enzymes are found in the peroxisomes of the majority of eukaryotic cells and catalyze the conversion of hydrogen peroxide to oxygen and water while employing manganese or iron as a cofactor. (Chelikani et al., 2004). The enzyme glutathione peroxidase (GPx), which has four selenium-based cofactors, catalyzes the breakdown of organic hydroperoxides and hydrogen peroxide. Animals include at least four distinct glutathione peroxidase isozymes. (Brigelius-Flohe & Traber, 1999).

Treatment with the amino acids arginine or glutamine can reduce the increased lipid peroxidation products (MDA) in rats given sodium nitrite. (El-Sheikh & Khalil, 2011). It has been demonstrated that administering marjoram oil in conjunction with sodium nitrite minimizes such hazards and lowers serum MDA levels. (Abu Aita & Mohammed, 2014). A considerable decrease in blood reduced glutathione, plasma total antioxidant capacity, glutathione-S-transferase, and a large rise in serum MDA were also brought on by rats ingesting sodium nitrite. Administration of aqueous green tea extract lessens the aforementioned alterations. (Gomma & Abd ELaziz, 2011).

Supplementing with garlic oil reduces the considerable rise in lipid peroxidation, the fall in glutathione levels, and the catalase activity that have been noticed in the liver and kidney of mice given sodium nitrite. (Abdul-Ameer & Abed, 2012). Additionally, it has been noted that in rats with liver damage caused by sodium nitrite, vitamin C and alpha-tocopherol dramatically reduce lipid peroxidation and boost SOD and catalase. (Ivanova et al., 2011). The goal of this study is to see if Schiff base produced from methionine can help reduce the adverse effects of sodium nitrite poisoning on oxidative stress markers and renal tissue in rats.
Materials and Methods

Experimental Animals

(40) young male and female rats (Rattus norvegicus) weighing around (350 gm± 25) with ageing were used in this investigation (14 -16 weeks). They were kept in hygienic circumstances. Before the application of experimental techniques, the animals were habituated to the laboratory environment for about 10 days.

Preparation of Methionine-Schiff Base

Azzouz and Ali (Azzouz and Ali 2010) described a technique for preparing and classifying methionine-schiff base. Salicylaldehyde or benzaldehyde, amino acid (methionine was chosen in this study), and sodium hydroxide (NaOH) are commonly mixed in ethanol.

Experimental Design

After 10 days of acclimatization, Forty adults male rats of about 4 months age, weighing about (350 ± 25g) were used in this experiment. Rats were divided randomly into 5 equal groups (8 rats in each group) and treated as following:

1. Control group: In which rats were given olive oil (0.2 ml/day). injected intraperitoneally (IP) (two times weekly for 6 weeks).
2. NaNO2 group: In which rats were given olive oil (0.2 ml/day) and 0.2% NaNO2 injected intraperitoneally (IP) (two times weekly for 6 weeks). (Krishnan et al., 2011).
3. Methionine-schiff base and NaNO2 group: In which rats were given (48 mg/kg) in 0.2ml olive oil and 0.2% NaNO2 injected intraperitoneally (IP) (two times weekly for 6 weeks) (Wasfi et al.,2015).
4. Methionine-schiff base and NaNO2 group: In which rats were given (96 mg/kg) in 0.2ml olive oil and 0.2% NaNO2 injected intraperitoneally (IP) (two times weekly for 6 weeks).
5. Methionine-schiff base group: In which rats were given (48mg/kg) in 0.2ml olive oil injected intraperitoneally (IP) (two times weekly for 6 weeks).

Samples collection

Rats were sacrificed at the end of experimental period (6 weeks). After light chloroform anesthesia, a Y shaped cut in the rat abdomen was done, blood and kidney are taken as following: kidney specimens from all experimental animals and fix them in 10% neutral buffer formalin for 24 hours, followed by dehydration in increasing concentrations of ethyl alcohol (70-100 percent), clearing with xylene, and embedding in paraffin wax (Suvarna et al., 2018) described sectioning approximately (5 m) thickness sections and staining them with hematoxylin and eosin (H&E) stain. Blood was collected directly from the heart by 10 ml disposable syringe of 22G needle (Parasuraman, et al., 2010). (3 ml) blood transferred into plain tube and centrifuged at 3000 rpm for 15 minutes to obtain the serum which then transferred into Eppendorf tubes and
stored at -20°C till used for measurement of different parameters (Cray et al., 2009).

**Statistical analysis**

Statistical Package for Social Sciences, Computerized SPSS (V.13) database were used for analysis of results of the current study. The statistics were presented as mean ± standard deviation (mean ±SD). Least significant difference test (LSD) was used to exam the change means (groups); P≤ 0.05 was reflected significant (SPSS, 2001).

**Result and Discussion**

The effect of methionine-schiff base, sodium nitrite and methionine-schiff base plus sodium nitrite on Serum malonaldehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase are Shown in table 1. The data revealed a significant (P>0.05) increase in Serum MDA, while catalase, GPx and SOD were significantly reduced (P<0.05) in NaNO2 group compared with control. Changes produced by NaNO2 on serum MDA, SOD and catalase are inverted after rats treated with methionine-schiff base (48 and 96 mg/kg) and their parameters became near to the control standards. In addition, Serum GPx is still significantly (P<0.05) lower than in control group. On the other hand rats given lower dose of methionine-schiff base (48 mg/kg), MDA was decreased, whereas SOD was increased compared with NaNO2 group but their values still significantly different (P<0.05) from the control values, furthermore, serum GPx and catalase were not affected and remained low as in the NaNO2 group. Whereas, Serum catalase SOD, MDA, and GPx were not affected when methionine-schiff base is managed individually related with control group (table 1).

Our study registered the histological changes in the nephrons of control group’s kidneys revealed a conventional glomerulus with ordinary podocytes and thin glomerular basement membrane, which was bordered by normal Bowman’s capsule. The epithelial organization of the proximal and distal convoluted tubules is regular (figure 2). The second group’s nephrons showed varying degrees of damage due to the use of sodium nitrite for 42 days (figure 3 & 4). When compared to the kidneys of the control group, the adverse effect of sodium nitrite on this group’s kidney was obvious (figure 2). Necrosis and bleeding of urinary tubules, as well as necrosis and/or atrophied glomeruli, were observed under a microscope (figure 3 & 4).

The third to fifth group, which was injected with methionine-Schiff base and sodium nitrite or methionine-Schiff base alone, showed that the damage was less severe than that induced by sodium nitrite, with a portion of the tissues resembling normal tissues. Microscopic examination reveals normal glomeruli and renal tubules, as well as a few degenerative tubule epitheliums (figure 5 & 6). It has been reported that Treatment with methionine were found to induce a protection against renal toxicity. The mechanism by which these agents can induce these protective effects may be due to their role in restoring the reduction of renal glutathione (GSH) level. Moreover, methionine undergoes several
biochemical reactions involved in the biosynthesis of cysteine, which has a protective role in detoxification mechanism. (Sonia et al., 2001). Furthermore depletion of GSH and increased cell peroxidation has been suggested to implicate the pathogenesis of renal damage and inhibition of renal phospholipase A2 and glutathione synthesis in rat induces an acute renal failure taken together, the protective effect of methionine has been known to be related to the amino acid’s antioxidant activity, when serving as glutathione precursor, indicated by an increased GSH level. (Panel et al., 2005; Derakhshanfar et al., 2009)

These effects of methionine derivative show the preservation of endoplasmic reticulum function against toxicity in the liver. The protective effect was due to its antioxidant property, which was revealed by reduced oxidative stress and enhanced functions of the endogenous antioxidative system (SOD, catalase, (GPx), (GST), (GSH), vitamin E and C) against intoxication (Periyasamy et al., 2017)

Methionine is capable to prevent fat accumulation in the liver and play a vital role in the detoxification of metabolic wastes and toxins. In addition, S- adenosyl methionine (SAM) is used to improve and normalize liver function. (Al-Saedi, J. 2016)

![Figure 2: tissue section of kidney from control group (H&E Stain, 100X). Normal glomerulus (1), Normal proximal tubules (2) and Normal distal tubules (3)](image)

Table 1: Effects of NaNo2 and Methionine-Schiff base on serum malonaldehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (μM/L)</th>
<th>GPx (μM/L)</th>
<th>SOD (μM/L)</th>
<th>Catalase (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.36±0.31</td>
<td>84.6±13.0</td>
<td>30.9±3.3</td>
<td>2.70±0.23</td>
</tr>
<tr>
<td>NaNo2 (0.2%)</td>
<td>2.69±0.5</td>
<td>43.4±7.6</td>
<td>19.9±1.0</td>
<td>1.58±0.21</td>
</tr>
<tr>
<td>Methionine-Schiff base (96 mg/kg) + NaNo2 (0.2%)</td>
<td>1.60±0.27</td>
<td>72.6±6.5</td>
<td>26.9±4.2</td>
<td>2.53±0.37</td>
</tr>
<tr>
<td>Methionine-Schiff base (48 mg/kg) + NaNo2 (0.2%)</td>
<td>1.73±0.20</td>
<td>52.5±11.5</td>
<td>25.8±2.0</td>
<td>1.80±0.47</td>
</tr>
<tr>
<td>Methionine-Schiff base (96 mg/kg)</td>
<td>1.35±0.10</td>
<td>74.3±9.2</td>
<td>29.5±4.9</td>
<td>2.56±0.51</td>
</tr>
</tbody>
</table>

LSD 0.35 11.0 5.0 0.74

Data are expressed as mean ± SD (n=8). Different letters indicate significant difference at (P≤0.05)
Figure 3: tissue section of kidney from group injected by Sodium nitrite (H&E Stain, 100X). Absence and shrinkage of glomerulus (1), hemorrhage and necrosis of urinary tubules (2)

Figure 4: tissue section of kidney from group injected by Sodium nitrite (H&E Stain, 400X) completely Absence glomerulus (1), necrosis of urinary tubules (2) congestion of glomeruli (3) and absence of bowman’s space (head arrow)

Figure 5: tissue section of kidney from group treated by Methionine-Schiff base and NaNo2 (H&E Stain, 100X). appearance of normal glomeruli (1) and renal tubules (2) and few degenerative of epithelium tubules (3)
Figure 6: tissue section of kidney from group treated by Methionine-Schiff base alone (H&E Stain, 400X). regular appearance of glomeruli (1) surrounding by bowman’s capsule with cleared bowman’s space (head arrow), normal renal tubules (2) and few degenerative of epithelium

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