Original Research Article

The potential hepatic and renal toxic effects of sodium glutamate and sulfite sodium in broiler chickens

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Abstract

Monosodium glutamate (MSG) and sulfite are frequently used as flavor enhancer and most applied as food additives in modern nutrition globally. This study was planned to investigate the toxic effect of MSG on liver and kidney when administered to broiler chickens during the growth period. Forty, day-old, unsexed Ross broiler chicks, assigned into 4 groups: (10 chicks each), fed on standard diet mixed with 0.75g of MSG/kg (group A), sodium metabisulfite 3.5g/kg (group B), 0.75g MSG + 3.5g sulfite /kg (group C) and control group (group D). Oxidative stress indicators malondialdehyde (MDA) and superoxide dismutase (SOD) activities were determined. Liver and kidney function tests for alkaline phosphatase (ALP) enzyme and creatinine metabolite were examined. Histopathology of liver and kidney tissues were conducted in all exposed groups. The results indicated increase in the levels of serum ALP, creatinine, MDA and SOD in all exposed groups in comparison with control. Disturbance in hepatic architecture with hydropic changes in hepatic cells with congestion of the interstitial blood vessels and necrobiosis of renal tubular epithelium were also registered.

Keywords: Monosodium glutamate, Meta bisulfite, SOD, MDA, Lipid peroxides, Liver, Kidney, Broiler.

Introduction

Flavor enhancing additives could be of great benefit in accessing the inherent nutrients of resultant feeds. MSG is regarded as an additive to enhance the palatability of food (Khalil and Khedr, 2016; Olarotimi, 2020). MSG is the sodium salt of glutamic acid, the most abundant naturally non-essential amino acids. It acts as a flavor enhancer in various food products, especially Chinese and South Asian dishes (López-Miranda et al., 2015). The perception of taste includes five basic tastes: saltiness, sweetness, sourness, bitterness, and newly discovered umami taste. MSG is considered one of umami-tasting compounds that added to food in various quantities to make it more appetizing and palatable (Yan et al., 2013). However, the excessive dosage of MSG administration has been implicated in conferring varying negative effects on animals (Eweka and Om’iniabohs, 2007). Diniz et al. (2004) reported that chronic administration of MSG induced oxidative stress in the tissues of young rats. Further study has also improved that MSG induced hyperglycemia caused oxidative stress in the kidney through the formation of free radicals and altered the antioxidant reactions mediated by reactive oxygen species (ROS) scavenging enzymes (Koya et al., 2003). Food additives have been associated with adverse health effects, especially on children, like obesity, hypertension, and attention deficit syndrome (Albus, 2012). It has been suggested that an increased intake of processed food containing MSG may be linked to the current increase in obesity and metabolic syndrome (López-Miranda et al., 2015). MSG is safe
if it is consumed at low doses; however, it has toxic effect on various body organs at high doses (Babuin and Jaffe, 2005). It has been reported that MSG exerts tissue-specific toxins and oxidative stress in various body organs. This oxidative stress has a role in the initiation and progression of cardiovascular diseases (Paul et al., 2012; Hazzaa et al., 2020). Furthermore, chronic oral MSG intake in rats was equally reported to have led to changes in antioxidant systems and renal markers including lipid peroxidation by products (Paul et al., 2012; Hazzaa et al., 2020). Sulfite is generally used in the form of sulfur dioxide or inorganic sulfite capable of producing sulfur dioxide (Qu’ D et al., 2017). Sulfite is a compound commonly used as a preservative in foods and pharmaceutical preparations (Vandevijvere et al., 2010). Sodium metabisulfite, potassium metabisulfite, sodium hydrogen sulfite, potassium sulfite and sodium sulfite are five sulfites absorbed by the gastrointestinal tract and distributed to all organs including the brain (Wang et al., 2016). Clinical observations have suggested that sulfite, if the body has excessive intake, can damage the gastrointestinal tract and liver, and cause symptoms such as dyspnea, diarrhea, vomiting, and cause reduction in red blood cells and hemoglobin (Jianying et al., 20 13). Studies have shown that sulfite is an important risk factor for the development and progression of liver disease (Niknahad and O’Brien, 2008). Liver, kidney, and heart have been reported to have high sulfite oxidase activity (Wee Hong et al., 2003). The liver is the most susceptible organ for drug-induced toxicity, probably because it is the main metabolic site of most drugs (Luis et al., 2007). Sodium sulfite (Na2SO3) is the main residual substance in traditional food and pharmaceutical products after sulfur fumigation. It is widely used as an additive for various dried fruits (such as pistachios, apricot), pharmaceutical products and alcoholic beverages (Gunnison, 1981). Sulfur dioxide can be converted to sodium sulfite after inhalation through the respiratory tract; eventually the compound circulates through the bloodstream into other organs, including the liver and kidneys (Mitsuhashi et al., 2001). It has been confirmed that high concentrations of sulfur dioxide and its derivatives can cause damage to various organs of the body, not only the gastrointestinal tract and heart, liver, kidney, and other organ tissues, but also the bronchial tubes and lungs (Ziqiang, 2003). The objective of this study was to evaluate the potential adverse effect produced from the administration of the salts of MSG or sulfite or their mixture in the broiler chickens during the normal growth period. Also, to access acceptable and safe inclusion levels in broiler diets to enhance the palatability for optimum feed performance.

Materials and Methods

Chemicals

The test substance, Mono sodium glutamate (MSG) is white crystalline powder, fast-soluble in water was purchased from Alpha Chem Company, India, purity 99%. Sodium metabisulfite was purchased from El Nasr Pharmaceutical Chemicals Company, Egypt.

Birds and experimental design:

The experiment was conducted using forty, day-old, unsexed Ross broiler chicks obtained from Assiut University Farm. The experiment lasted for 6 weeks, and the chicks reared at the poultry unit of Animal Lab of the department forensic medicine and toxicology, at the Faculty of Veterinary medicine, Assiut University. Birds were fed broiler starter and finisher diets ad libitum from 0 to 4 weeks and 4 to 6 weeks, respectively. The experimental feeds were formulated to be isonutritive and isoenergetic, according to the nutritional requirements recommended by the lineage handbook and the vaccination, health rules and poultry management practices were maintained (Cruz et al., 2017).

On arrival of the chicks, they were weighed and assigned to the 4 dietary treatment groups: each group contain 10 broiler chicks: monosodium glutamate diet containing 0.75g of MSG/kg of feed (group A), sodium metabisulfite diet containing 3.5g/kg of feed (group B), mix diet is containing 0.75 g MSG + 3.5g sulfite /kg of feed (group C) and control group (group D).

Blood sampling

At the end of the experiment after 6 weeks, 10 birds per group were randomly selected for blood sampling. The birds were fasted overnight, and blood samples were collected from the wing veins into dry clean centrifuged glass tubes without any coagulant to separate the serum for determination of serum electrolytes and antioxidant status indicators. Blood samples were left for 15 min at room temperature, and then, the tubes were centrifuged for 10 min at 3000 rpm to obtain clean supernatant serum. The harvested serum samples were kept frozen at − 20 °C until the determination of serum ALP, creatinine, MDA, SOD, concentrations.
Organ’s collection
Liver and kidney tissue were dissected, and the samples were fixed in 10% formalin for histopathological examination.

2.5. The biochemical assay of liver and kidney function tests in broiler chickens
Alkaline phosphatase (ALP) was measured according to Rec (1972) and creatinine level was determined according to Sies et al. (1985) in serum using a spectrophotometer (Optizen 3220 UV, Korea).

2.6. Antioxidant status indicator measurement
Malondialdehyde (MDA)and Superoxide dismutase (SOD)
The determination of the serum MDA was done by thiobarbituric acid (TBA) assay method as described by Baliga et al. (2018), liver and serum superoxide dismutase (SOD) activity was determined as highlighted by Oyanagui (1984) using a spectrophotometer Optizen 3220 UV, Korea.

Histopathology
Liver and kidney tissue were dissected, and the samples were fixed in 10% formalin. Paraffin was used to stabilize the tissue and stained by hematoxylin and eosin (H&E) to examine the tissue under the light microscope.

Statistical analysis
All experimental data obtained were subjected to one-way analysis of variance (ANOVA) using Graph Pad Prism, software version 6.01. Significant differences between the treatment means were compared using Tukey’s honestly significant difference (HSD) option of the same software at 5% level of significance.

Results
Biochemical parameters related to the liver and kidney function in broiler chickens
Alkaline phosphatase
Glutamate exposed group showed a significant (P<0.05) increase than control, sulfite group and mixture group. Sulfite exposed group showed no significance with the control but showed a significant (P<0.05) decrease than both glutamate and mixture exposed group (Table 1).

Creatinine
All exposed groups, glutamate, sulfite and the mixture groups showed a significant (P<0.05) increase in the creatinine levels than control. No a significant (P<0.05) difference could be recorded between the different exposed groups (Table 1).

Table 1. Biochemical parameters related to the liver and kidney function in broiler chickens.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alkaline phosphatase (IU/L) (Mean ± SE)</th>
<th>Creatinine (mg/dl) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>86.62 ± 18.85</td>
<td>10.94 ± 2.88</td>
</tr>
<tr>
<td>Glutamate</td>
<td>107.59 ± 32.29 ab</td>
<td>12.88 ± 3.45*</td>
</tr>
<tr>
<td>Sulfite</td>
<td>48.56 ± 9.13ac</td>
<td>12.59 ± 1.77*</td>
</tr>
<tr>
<td>Mixture</td>
<td>97.96 ± 15.5 *ab</td>
<td>12.94 ± 1.39*</td>
</tr>
</tbody>
</table>

Oxidative status in liver and serum in broiler chickens exposed to Glutamate, sulfite and their mixture.

Superoxide dismutase assay
The activities of SOD in serum and the hepatic tissues showed a significant (P<0.05) increase in Glutamate, sulfite and the mixture exposed broilers in comparison to the control. There is a significant (P<0.05) difference between the different exposed groups (Table 2).

Table 2. Oxidative status in liver and serum in broiler chickens exposed to Glutamate, sulfite and their mixture.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Superoxide dismutase in liver (IU/L) (Mean ± SE)</th>
<th>Superoxide dismutase in serum (IU/L) (Mean ± SE)</th>
<th>MDA (mg/dl) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>298.125 ± 327.41</td>
<td>2031.1 ± 555</td>
<td>20.64 ± 2.88</td>
</tr>
<tr>
<td>Glutamate</td>
<td>363.018 ± 32.37 *bc</td>
<td>2396.3 ± 546b</td>
<td>36.58 ± 3.45 *bc</td>
</tr>
<tr>
<td>Sulfite</td>
<td>332.55 ± 10.36*ac</td>
<td>7338.4 ± 253*ac</td>
<td>38.59 ± 1.77 *bc</td>
</tr>
<tr>
<td>Mixture</td>
<td>331.82 ± 15.24*ab</td>
<td>1932.7 ± 685ab</td>
<td>40.54 ± 1.54*ab</td>
</tr>
</tbody>
</table>

MDA determination
Lipid peroxidation (LPO) was assessed by measuring MDA and 4HNE reactive substances levels in the serum samples of broiler chickens exposed to MSG, sulfite, and their combination at the end of experimental time. Table 2 recorded that these treatments caused a significantly (p<0.05) marked increase in the levels of MDA and 4HNE in blood when compared with control.

Histopathological investigation of liver and kidney of broiler chickens
Liver section taken from broilers treated with MSG represented in Fig.1 (a) and (b), showed hepatocytes damage was manifested by marked disturbance in hepatic architecture with fatty degeneration of hepatocytes and dilation of sinusoidal blood vessels, Liver section taken from sulfite group appeared in Fig 1 (c), showed diffuse congestion of the vasculature and fatty degeneration of hepatocytes and sinusoidal dilation. Liver section taken from (MSG+ sulfite) mixture group Fig.1 (d), revealed mild congestion of the vasculature.
Kidney tissues taken from broilers treated with MSG represented in Fig. 2 (a) and (b), showed congestion of the interstitial blood vessels. Kidney sections taken from sulfite group appeared in Fig. 2 (c) showed that congestion of the interstitial blood vessels and necrobiosis of renal tubular epithelium. Kidney sections from taken from group C (MSG+ sulfite) mixture group Fig. 2 (d) showing congestion of the interstitial blood vessels and necrobiosis of renal tubular epithelium.

**Discussion**

Glutamate exposed group showed a significant (P<0.05) increase in alkaline phosphatase concentration than control, sulfite group and mixture group. Sulfite exposed group showed no significance with the control but showed a significant (P<0.05) decrease than both glutamate and mixture exposed group. This result corroborated the findings of Tawfik and Al-Badr (2012), who recorded a significant increase in serum ALT in rats fed with 0.6 and 1.6 mg of MSG/g body weight. Okediran et al. (2014) also recorded a significant increase in serum ALT on rats fed with 1 g of MSG per day. Similarly, Gbore et al. (2016) reported a significant increase in ALT after administration of 2 mg and 4 mg of MSG/kg body weight to rabbits. The increase in serum content in ALT could suggest disturbances in metabolism affecting the liver function. Therefore, the increase in ALT activity might indicate the liver damage. Liver function tests showed a consistent rise in the alkaline phosphatase at all time-points, with a rise in the serum transaminases after 3 and 12 months of glutamate exposure in Wistar rats (Nnadozie et al., 2019). According to Tawfik and Al-Badr (2012), MSG could dissociate easily to release free glutamate and ammonium ion that could be toxic unless detoxified in the liver via the reactions of the urea cycle. Thus, the possible NH4+ overload that may occur as a result of an increased level of glutamate following MSG intake could damage the liver, resulting in enzyme leakage that might lead to observed elevation in their activities. The enzymes are released into the circulating blood only after damage to liver structural integrity (Janbaz and Gilani, 2000). However, the examination of the histological structure of the liver revealed no damage suggesting that ALP content increased without reaching the critical level indicating the hepatic cells damages, the results presented in the present study revealed that feeding broiler chickens with MSG improve growth performance with no detrimental effect on the histological structure of the liver (Ciza et al., 2019). This increase could also be explained by free radical production which reacts with polyunsaturated fatty acids of cell membrane leading to impairment of mitochondrial and plasma membranes resulting in enzyme leakage. The result seemingly agrees with the reports of (Farombi and Onyema, 2006; Onyema et al. 2012). In our work, biochemical assay demonstrated the changes in liver enzymes and products of oxidative
stress. The activities of SOD in serum and the hepatic tissues showed a significant (P<0.05) increase in glutamate, sulfite and the mixture exposed broilers in comparison to the control. There is a significant (P<0.05) difference between the different exposed groups. The exposed groups exhibited high statistically significant increase in liver enzymes and serum MDA compared to the control group. Nnadozie et al. (2019) and Hussain et al. (2020) documented that the activity of serum liver enzymes increased in male rats that were fed MSG probably due to MSG induced oxidative stress in the liver. Thus, it could be concluded that MSG may be hepatotoxic at a low dose. The serum ALP shows the functional activity of liver. An increase in the activities of these enzymes indicates a toxic effect of MSG exposed doses. In this study, the exposed groups, glutamate, sulfite and the mixture groups showed a significant(P<0.05) increase in the serum creatinine levels than control. There is no significant (P<0.05) difference could be recorded between the different exposed groups. The present result supported the findings of Khaleda et al. (2009) who reported a significant increase of urea in broiler chickens supplemented with 0.5 and 1% MSG. Inuwa et al. (2011) also reported an increase in serum concentration in urea in rats fed with 200, 300 and 400 mg MSG/kg body weight. Sharma et al. (2013) who observed cases of lithiasic kidneys (hydronephrosis) and urinary tract obstruction in rats with 2 mg MSG/kg of live body. In the same context, Airaodion, et al., (2020) declared that the elevation of renal indices by MSG is an indication that it is nephrotoxic. However, Ciza et al. (2019) stated that the exploration of histological sections of the kidneys of chickens fed on MSG revealed no mark of injury but there is an increase in urea level could indicate an impaired of the kidney function after MSG exposure.Similarly, Hussin et al. (2021) found enlargement in a mesangial mass represented by hypertrophy and hyperplasia of mesangial cells leading to mesangial proliferative glomerulonephritis which accompanied with an increase in creatinine values, indicating a disturbance in renal function. This suggested that MSG intake leads to indirect narrowing of the glomerular capillary lumen, resulting in renal filtration disorders and causing renal failure. After sulfite administration in Wistar rat, there was an increase in biochemical parameters urea, creatinine, uric acid, liver transaminases (El Kadi et al., 2014). Sulfite induced significant increase in serum activity of AST, ALT, and ALP as well as the serum urea and creatinine levels in the treated rats (Mahmoud et al., 2015). In the histological examination, the glutamate, sulfite, and the mixture groups showed the altered liver architecture, congestion in CV, dilated sinusoids, and decreased the size of hepatocyte nucleioids. Kidney tissues taken from broilers treated with MSG showed congestion of the interstitial blood vessels. The kidney in sulfite group appeared with slight congestion of the interstitial blood vessels and necrobiosis of renal tubular epithelium. Similar findings were observed by Hamad and Hamed, (2020); Hussain et al. (2021). In similar study, MSG treated animals revealed prominent areas of severe vaculated hepatocyte with pyknotic nuclei, marked dilated central vein congested with hemolysis blood cells and perivascular inflammatory cells infiltrations. Histopathological examination of the experimental animals indicated little sinusoidal dilatation in rats treated with 200ppm of Na 2 SO 3. Hepatic vacuolation, large sinusoidal dilatation, degenerative changes and cellular congestion were shown in liver of rats treated with 500 and 1000ppm of Na 2 SO 3 when compared to the control group. Administration of Na 2 SO 3 to rats exhibited serious effects on both liver and kidney cells (Mahmoud et al., 2015).

Conclusion

It was concluded that the administration of the salts of MSG or sulfite or their mixture in the broiler chickens during the growth period causes deterioration in different biochemical measurements, activities of antioxidant enzymes, liver and kidney functions and deterioration of liver and kidney tissues and has the affection on the capacity of antioxidant and increases the activities of lipid peroxidases products. This study could be suggested further investigation be carried out to understand the effects food additives in long administration.

References


