Ameliorating Effect of Moringa against Liver and Kidney Injury Induced by Monosodium Glutamate

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RME and ASA designed the study, performed the statistical analysis, wrote the protocol, managed the analyses of the study and wrote the first draft of the manuscript. Author TIA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Monosodium glutamate (MSG) produces adverse and damaging effects in different organs like liver and kidneys. Moringa has ameliorating effect on kidney and liver injury induced by monosodium glutamate.

Objective: To study the ameliorating effect of moringa against rats liver and kidney injury induced by monosodium glutamate.

Design: Prospective study.

Setting: College of Pharmacy, Qassim University.

Materials and Methods: This study was performed on 20 male rats and equally divided into 4 groups. The first group was control group, second group was moringa group, third group was MSG group and forth group was MSG plus moringa group. We determined liver function, albumin, total protein, kidney function, electrolytes and histopathological examination of tissue.

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Main Outcome Results: Moringa has ameliorating effect on kidney and liver injury induced by monosodium glutamate.

Sample Size: A total of 20 male rats.

Results: There was a significant increase in the levels of serum aspartate transaminase (AST) and alanine transaminase (ALT), alkaline phosphatase (ALP), urea and creatinine. Significant decrease in the levels of albumin, total proteins and sodium levels in rats treated with monosodium glutamate. Kidney sections revealed normal structure of glomeruli and renal tubules as control group, liver revealed good improvements and mild cellular infiltrations were observed in rats treated with MSG and moringa group.

Conclusion: Moringa causes ameliorating effect on kidney and liver injury induced by monosodium glutamate in rats.

Limitation of the Study: Few studies about the protective effect of Moringa against toxic effect of MSG. So we need to focus on its beneficial effect against toxicity induced by MSG.

Keywords: Monosodium glutamate; liver; kidney; Moringa oleifera.

1. INTRODUCTION

Glutamate is found as a natural product as part of the environment. It is also a product released by humans that plays a crucial part in our bodies metabolism. Many tissue peptides and proteins are mostly made up of glutamate. Consequently, many foods that contain a large amount of protein also consist mostly of glutamate. Examples milk, cheese, meat, fish and vegetables (Tomato and mushrooms) [1].

Monosodium glutamate (MSG) is commonly used as a food additive for flavour enhancer particularly in West African and Asian dishes [2]. MSG is accepted as a safe food additive when used at small quantity [3]. However, if used without labelling, it can cause abuse of food that contain high amount of MSG [4]. The general adverse effects of consuming large amount of MSG are numbness at the back of the neck with radiation to the arms and neck, weakness and palpitations [5].

Previous studies demonstrated the resultant negative impact of MSG as seen in organ destruction including organs such as the brain, liver, kidneys and also the thymus in experimental animals [6-8]. When tested on rats, a response of oxidative stress is noted that can be observed by the continuous change in the resultant reaction from an enzymatic to a non-enzymatic oxidative reaction. This takes place in a lot of organs including the kidneys, brain and liver [9]. Various studies have designated MSG as a poisonous product for both animals and humans alike [10-11].

Various signs are thought to manifest as a result of overconsumption of MSG. These include faintness, reddening, numbness, giddiness, perspiration and headaches. Intake of MSG is also thought to result in or aggravate a number of disorders e.g. urticaria, neuropathy, ventricular arrhythmia, asthma, atopic dermatitis and stomach aches [12].

The consumption of high levels of MSG can lead to extreme break down of glutamate by the kidneys. When tested in experimental rats, the rats that were administered MSG at high levels showed higher levels of peroxidation of lipids, higher levels of free radicals and lower amounts of enzymes known to act as anti-oxidants [13-14]. Moreover, exposure of kidney culture cells to critical levels of glutamate has been proven to cause serious toxicity [15]. The renal system is at risk from destruction as a result of oxidative stress because of the high quantities of long chain polyunsaturated fatty acids that they contain [16].

Moringa oleifera or M. oleifera is a member of the Moringaceae family. It is known to contain high amounts of substances that humans require as well as possessing a range of phytochemicals in various parts including leaves, pods and seeds. Elements that are in high supply include: seven times greater vitamin C levels compared to oranges, a more than 10 times greater level of vitamin A when compared to carrots, 17 times greater calcium than milk, 15 times greater potassium level compared to bananas, nine times greater level of protein when contrasted with yoghurt and finally 25 times more iron than spinach [17].

Antioxidants are also in high supply in Moringa [18]. Examples of these include glutathione and ascorbic acid — both present at great levels in the chloroplasts of moringa as well as in different
parts of the cell and also play a significant part in oxidative stress [19].

In South Asia, practically every part of the plant has been used to treat domestic illnesses including GI tract, blood, hepaticrenal and cardiovascular illnesses [20].

One current study determined that the components extracted from the moringa leaf can have a major effect on the therapy of toxic liver disease and iatrogenic. When large amounts of MSG were consumed, use of the extract of the moringa leaf in therapy led to the management of various irregularities found in the kidney and the liver which was attributed to the decrease in enzymatic action and the evident enhancement in levels of oxidative stress, antioxidant factors, biochemical factors and damage caused to either the liver or kidney [21].

Recently, researchers had panicked about the evident rise in the negative impacts and toxic effects of MSG. Particularly as there were only a small number of publications covering histological and biochemical research into injury caused to the liver and kidneys over the course of testis treatment in experimental animals when MSG was used [22-23].

The primary aim of this study is the examination of the impact of moringa and the part they play in improving MSG-induced induced toxicity seen in the malfunctioning of the kidneys and the liver.

2. MATERIALS AND METHODS

2.1 Chemical and Drug

- 100 mg/kg of MSG (monosodium glutamate).
- Qingdao Huifenghe MSG Co., Ltd, in China supplied moringa at 100 mg/kg.

2.2 Animal

Albino Sprague Dawley rats were used in this study. The rats were all males, there were 20 of them and they each weighed between 200-250 g. The environment that the animals were kept in was managed. The temperature was maintained at 23±2°C, humidity at 55±5%, with a 12hour light to dark cycle as well as the provision of their diet and water that they had access to all the time. The therapeutic period encompassed four weeks during which the animals were observed closely. Moreover, the animals' consumption of food and water was documented on a weekly basis throughout the entire period of the experiment.

2.3 Experimental Design

Initially, the rats were left for a week to acclimatise to their surroundings. This was followed by their separation into four equal groups that each contained five rats.

G1: The no treatment control group.
G2: Designated the moringa group. This group was given moringa using an oral gavage at 100 mg/kg of the animal's body weight. This lasted for four weeks according to Berkovich et al. [16].
G3: Designated the MSG group. These rats consumed monosodium glutamate orally at gavage 100mg/kg of body weight for a period of four weeks according to Egbuonu et al. [3].
G4: This group was given both MSG and moringa over a four week period by oral gavage.

2.4 Blood Sampling

At the end of the four weeks the treatment period had come to an end and the rats were euthanised using sodium pentobarbital via intraperitoneal injection. The inferior vena cava of each rat was used as the site for blood collection. This was collected in non-heparinised tubes and placed at room temperature for a period of half an hour until clot formation. Samples were centrifuged at 5000rpm for a period of 10 minutes. The serum was drawn out of the tube and frozen as aliquots in the -80°C freezer for later use. Before testing samples, the sample would be left at room temperature to thaw.

2.5 Tissue Sampling

Renal and hepatic tissue samples were removed from all rats and formalin fixed (10%) prior to their placement in a glass vessel. These were then H and E stained for histopathological analysis.

2.6 Biochemical Assays

2.6.1 Liver function biomarker

The protocol established by Reitman and Frankel (1957) was used to determine the activity of AST and ALT in the serum. This methodology was colorimetric [24]. A commercial developed kit detailed in Belfield and Goldberge (1971) was utilised to determine ALP activity in the serum. Commercial kits were also used to determine quantities of total protein and albumin in the
serum as noted in Bowers and Wong (1980) and Doumas et al. (1977), respectively [25].

2.6.2 Kidney function biomarker

A commercial kit (Diamond from Egypt) was employed to assess the quantities of creatinine and urea in the serum as detailed in Henry et al. (1974) and Patton and Crouch (1977), respectively [26]. The electrolytes potassium, sodium, calcium and chloride ions in the serum were all determined by employing a commercial kit (Sensa core electrolyte from India) and this was conducted as detailed in Tousson et al. (2018) [27].

2.6.3 Histopathological investigation

To prepare the tissues for histopathological examination, following euthanisation the rats kidneys and liver were removed quickly. This was followed by a triple wash in saline kept at frozen levels and then dabbed on filter paper. 10% buffered neutral formalin solution was used to fix the kidneys and they were kept in this for a total of 48hrs before being prepared for paraffin sectioning. In keeping with the protocol described in Bancroft and Cook (1994) the tissue sections were prepared using the H and E stain [28].

2.7 Statistical Analysis

SPSS version 21 was the statistical software employed for examination of the findings. The data generated was presented as mean ± standard deviation of mean. This was calculated using one way analysis of variance (one way ANOVA) which allowed the establishment of whether significant differences existed between treatment groups. Regarding the biochemical information gathered, a value at p <0.05 was considered to be statistically significant.

3. RESULTS

3.1 Changes in Liver Functions

As illustrated in Table 1, rats administered MSG depicted much higher levels of ALT, AST and ALP compared to rats administered M. oleifera or the two control group; G1 and G4. Contrastingly, amounts of total protein and albumin measured fell greatly for the MSG group compared to the M. oleifera and control groups as depicted in Table 1. Finally, the G4 group which was administered both MSG and M. oleifera showed an adjustment in these liver function factors balancing between the MSG group and the other two groups (Table 1).

3.2 Histopathological Changes in Liver

Fig. 1A and 1B depict the hepatocyte morphology for the control group (G1) and the M. oleifera group (G2) respectively. The hepatocytes imaged appear polygonal and enclose eosinophilic cytoplasm and nuclei that are round and conspicuous. In the space between the hepatic cords an organisation of hepatic sinusoids is identified together with proper organisation of Kupffer cells. Fig. 1A and 1B therefore illustrate normal hepatocyte morphology.

Fig. 1C is an illustration of the liver tissue section of the G3 MSG treated group of rats. The hepatocytes show the formation of vacuoles within the cytoplasm, atrophied. They also depict modest degree of infiltration of the cells. The blood sinusoids also present low level dilations and congestion. This therefore is evidence of hepatotoxicity. Fig. 1D showed liver tissue sections from group G4 which was treated with both MSG and M. oleifera. Hepatotoxicity was

<table>
<thead>
<tr>
<th>Groups</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT(U/I)</td>
<td>32.45 ± 2.34</td>
<td>29.05 ± 1.65</td>
<td>58.60 ± 2.83</td>
<td>34.28 ± 2.71</td>
</tr>
<tr>
<td>AST(U/I)</td>
<td>110.4 ± 6.57</td>
<td>103.0 ± 9.14</td>
<td>166.5 ± 9.14</td>
<td>117.5 ± 8.26</td>
</tr>
<tr>
<td>ALP(U/I)</td>
<td>141.5 ± 7.95</td>
<td>140.9 ± 11.01</td>
<td>216.0 ± 12.46</td>
<td>150.5 ± 9.91</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.61 ± 0.18</td>
<td>4.88 ± 0.18</td>
<td>3.25 ± 0.12</td>
<td>4.38 ± 0.18</td>
</tr>
<tr>
<td>T.protein (mg/g)</td>
<td>6.04 ± 0.72</td>
<td>6.40 ± 0.55</td>
<td>5.13 ± 0.29</td>
<td>5.99 ± 0.34</td>
</tr>
</tbody>
</table>

The data is represented as mean ± SD. Groups listed include control (G1); M. oleifera(G2); MSG (G3); MSG + M. oleifera (G4). Alanine aminotransferase assay (ALT); Aspartate aminotransferase assay (AST); Alkaline phosphatase assay (ALP)

*(P<0.05) a significant level of difference from the control group

*(P<0.05) a significant level of difference from the MSG group
present in these cells but at milder levels compared to the G3 MSG group. Atrophy was mild. Low levels of vacuole formation in the cytoplasm were noted and only a few cellular infiltrations were noted.

### 3.3 Changes in Kidney Functions and Electrolytes

Table 2 listed the results of the creatinine and urea levels in the serum of rats and demonstrated that significantly higher levels were associated with MSG treatment when contrasted with controls. This is dissimilar to the findings of the sodium ions in the serum as presented in Table 3 and which registered significant reductions in levels in the MSG group compared to the control and *M. oleifera*.

Moreover, Tables 2 and 3 also present the results of the G4 group treated with both *M. oleifera* and MSG and which showed improvements of liver function compared to the MSG group through regulation of the electrolytes.

### 3.4 Histopathological Changes in Kidney

Fig. 2A and 2B presented normal renal morphology for the control G1 group and the *M. oleifera* G2 group. Histological appearance of the glomeruli and renal tubules was normal in both the medullary and cortical segments. Inflammation was absent in all sections including the Bowman’s capsule and the glomerulus it surrounds as well as both the distal and proximal tubules.

Fig. 1A-1D. Illustrations of sections of rat liver presented as photomicrographs. Sections are H and E stained for morphology analysis. 1A and 1B represent the control group and the *M. oleifera* group, respectively. They both depict normal hepatocyte morphology (hp) and structure of the central vein (C). 1C illustrates the tissue sections of the liver of rats treated with MSG demonstrating severe atrophy of hepatocytes, blood sinusoids depicting congestion and modest levels of infiltration of the cells (arrows). 1D illustrates liver tissue sections from rats treated with both MSG and *M. oleifera*. These demonstrated low levels of infiltrations of the cells (arrows)
Table 2. Variations in the kidney function parameters across the different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.61±0.08</td>
<td>0.47±0.02</td>
<td>1.12±0.10</td>
<td>0.89±0.05</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>25.71±1.15</td>
<td>25.22±1.30</td>
<td>39.08±1.73</td>
<td>33.50±1.89</td>
</tr>
</tbody>
</table>

The data is represented as mean ± SD. Groups listed include control (G1); M. oleifera (G2); MSG (G3); MSG + M. oleifera (G4)
* (P<0.05) a significant level of difference from the control group
# (P<0.05) a significant level of difference from the MSG group

Fig. 2A-2D. Illustrations of sections of rat kidney presented as photomicrographs. Sections are H and E stained for morphology analysis. 2A and 2B represent the control group and the M. oleifera group, respectively. They both depict normal renal morphology illustrating various structures of the renal cortex including the renal corpuscles (G) and both convoluted tubules (distal and proximal). 2C illustrates the tissue sections of the kidney of rats treated with MSG demonstrating modest atrophy, destruction of the glomeruli, vacuole formation and infiltration of cells (black arrows) and areas of degeneration (white arrows). 2D illustrates kidney tissue sections from rats treated with both MSG and M. oleifera. These demonstrated normal morphology as evidenced from the kidney tubules and kidney corpuscles (G)

Table 3. Variations in levels of electrolytes across the groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium ions (mmol/l)</td>
<td>3.75±0.28</td>
<td>3.55±0.27</td>
<td>5.29±0.61</td>
<td>4.11±0.35</td>
</tr>
<tr>
<td>Sodium ions (mmol/l)</td>
<td>137.1±9.17</td>
<td>135.5±5.56</td>
<td>130.3±7.55</td>
<td>136.5±10.48</td>
</tr>
<tr>
<td>Calcium ions(mg/dl)</td>
<td>1.18±0.020</td>
<td>1.18±0.013</td>
<td>1.19±0.025</td>
<td>1.19±0.021</td>
</tr>
</tbody>
</table>

The data is represented as mean ± SD. Groups listed include control (G1); M. oleifera (G2); MSG (G3); MSG + M. oleifera (G4)
* (P<0.05) a significant level of difference from the control group
# (P<0.05) a significant level of difference from the MSG group
4. DISCUSSION

Various countries use the flavour stimulating properties of monosodium glutamate (MSG) in their foods [2]. A number of symptoms have been associated with its ingestion including loss of feeling at the back of the neck which transmits to the remainder of the neck and arms, as well as palpitations and feelings of faintness.[5] The Moringaceae plant family includes Moringa oleifera or M. oleifera which has powerful antioxidant properties [17-18]. Treatments associated with M. oleifera are varied and include treatments against cancer, diabetes, inflammation, oxidative free radicals and elevated levels of lipoproteins and blood pressure [21].

This research aimed to investigate the potential renal and hepatic adverse effects of MSG. The study also thought to establish the beneficial impacts of M. oleifera with respect to both renal and hepatic MSG-related damage.

This study determined that hepatic function indicators including ALT, ST and ALP were raised significantly in rats administer MSG when contrasted against the control and M. oleifera groups. Contrastingly, total protein and albumin levels fell to significant levels in MSG treated rats compared to the control group. These result support evidence from Oscar et al. (2006) who demonstrated MSG-related destruction of hepatocytes and higher levels of AST and ALT. These increases in levels of AST and ALT can be reduced when treated with M. oleifera which appears to manifest a protective influence on the permeability of cell membrane which is attributed to its targeting of free radicals and oxidants [29].

The enhancing effects of M. oleifera on both the kidney and renal parameters including ALT, AST, ALP, creatinine, urea and bilirubin was investigated in a study by Suleiman and colleagues who examined its leaf extract impact and deduced lower levels of these metabolites together with lower levels of albumin and total protein [30]. The beneficial therapeutic effects of the leaf extract of M. oleifera on liver diseases causing adverse effects and iatrogenic was demonstrated by Albrahim and colleagues. This analysis demonstrated that the toxic effects of MSG were successfully treated with M. oleifera. This was demonstrated by the lower enzyme levels, biochemical improvements and improvements in anti-oxidant behaviour in relation to oxidative stress and liver damage [22].

Regarding renal function, this study demonstrated significantly higher levels of urea and creatinine in the serum of MSG-treated rats when compared to the control group. Contrastingly, significantly lower levels of sodium ion in the serum were found in MSG treated rats when contrasted against the control group. Moreover, significant variation was found in calcium electrolyte levels in the serum of MSG-treated rats when compared with the M. oleifera and the control group. Oxidative stress was also investigated by changes in both the enzymatic and non-enzymatic oxidative reactions in renal tissue resulting in the manifestation of nephrotoxicity [11]. This study demonstrated the protective properties of M. oleifera brought about through its targeting of antioxidants including glutathione and ascorbic acid which were detected at raised levels in the chloroplasts and other sections of M. oleifera and that demonstrate the significant part they play in oxidative stress [19].

This research demonstrated normal hepatic morphology of tissue sections from the M. oleifera group and the control group with polygonal hepatocytes with conspicuous nuclei that are round in shape, eosinophilic cytoplasm, the arrangement of hepatic sinusoids in areas between the hepatic cords together with proper organisation of Kupffer cells.

Contrastingly, the hepatic tissue sections of MSG-treated rats showed levels of hepatotoxicity which appeared as modest to severe levels of atrophy, vacuole formation in the cytoplasm, modest infiltration of cells, low levels of blood sinusoid congestion and dilation. These effects appeared reduced in rats treated with both MSG and M. oleifera with lower levels of atrophy, vacuole formation in the cytoplasm and infiltration of cells. This data supported a study by Thomas and colleagues which determined...
that various hepatic cells were adversely impacted together with negative effects on metabolism when treated with MSG [13].

Furthermore, renal sections from the M. oleifera and the control group were both shown to have normal morphology represented by examination of the glomeruli and kidney tubules of the medullary and cortical sections. Inflammation was absent from the Bowman's capsule and the glomerulus it surrounded as well as both convoluted tubules (proximal and distal). Injury of renal tissue was seen in the tissue sections from rats treated with MSG including the urinary tubules, glomeruli, modest infiltration of cells, degeneration and atrophy of kidney tubules and a decrease in lumen size. Moreover, focal necrosis and inflammation of regions showing cellular infiltration of interstitial tissues with monocytes and lymphocytes was also noted.

Renal sections from rats administered both MSG and M. oleifera presented normal morphology as depicted by the kidney tubules and the glomeruli when contrasted against the control. This study supports data derived from various studies including that of Tawfik et al. and Sharma and Thomas et al. by illustrating damage caused to both renal and hepatic sections by MSG [27,31].

In keeping with the results of Albrahim and colleagues, liver tissue sections from both the control and the M. oleifera groups presented normal hepatocytes that were polygonal in shape and of a large size, containing eosinophilic cytoplasm and conspicuous and rounded nuclei. Between the hepatic cords, regularly spaced hepatic sinusoids were organised with proper organisation of Kupffer cells.

Contrastingly, the tissue sections from the liver of the MSG-treated group showed signs of hepatotoxicity as evidenced by modest levels of damage of hepatocytes, a large number of cell foci depicting apoptosis, increased formation of vacuoles in the cytoplasm, congestion of blood sinusoids, conspicuous necrosis and disorganised arrangements of Kupffer cells between hepatocytes and around the portal region. This was dissimilar to the tissue sections from the group treated with both MSG and M. oleifera which presented with lower signs of hepatotoxicity as presented by low levels of vacuolisation of the cytoplasm, infiltration of the cells, congestion of blood sinusoids in both the portal and central regions and increased evidence of hepatocytes undergoing apoptosis [22].

This study supported findings of Albrahim and colleagues with respect to histopathology of liver tissue. One study by Sharma demonstrated the negative impact of MSG if peripheral body parts including the kidney with signs of higher lipid peroxidation, decreased presence of enzymes targeting oxidants and fibrosis of the tubule-interstitial parts. These are all manifestations of the impact of MSG consumption which provide further evidence for the fact that the MSG-related kidney toxicity being associated to higher levels of oxidative stress [22,24]. Another study by Tawfik et al. reported the changes instigated by MSG on renal and hepatic functions as well as body weight. The manifestation of such changes in both the liver and kidney are attributes to the roles of both these organs in the detoxification process of foreign substances entering the body [25,29].

5. CONCLUSION

This research has therefore illustrated the changes in both hepatic and renal function that are brought about by consumption of MSG. These changes manifest as a result of oxidative stress in renal and hepatic tissues that is caused by MSG and also because of the involvement of both the kidney and liver in the process of foreign substance detoxification. M. oleifera has been shown to protect against MSG destruction of tissues by its antioxidant properties. Thus, it can be deduced that M. oleifera can have beneficial impacts on the liver and kidneys by improving the damage to these organs that’s caused by MSG as observed in rats.

ETHICAL APPROVAL

This research respected the ethical guidelines that are outlined by the Committee of National Research Center of Saudi Arabia. The protocol utilized in this study was approved by the Animal Ethics Committee of the Faculty of Pharmacy at the Al-Qassim University (in Al-Qassim in Saudi Arabia).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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