THE INFLUENCE OF WATERMELON (CITRULLUS LANATUS) JUICE INTAKE ON THE KIDNEY OF WISTAR RATS (RATTUS NOVERGICUS) EXPOSED TO MONOSODIUM GLUTAMATE

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Introduction
Monosodium Glutamate (MSG) is a food additive used in processed food. Long time use of MSG causes oxidative stress and damages kidney. The lycopene content in watermelon is potential in reducing oxidative stress. This research aimed to discover the effect of watermelon juice in preventing kidney damage in rats. 30 Wistar rats were divided into 5 groups: four groups fed with 10 mg of MSG / g body weight; three groups of these also fed with 3 different concentrations of watermelon juice (25% in P1, 50% in P2, and 100% in P3) whereas the MSG only is as the control positive (KP). The fifth group is supplemented with water as the negative control (KN). Blood and kidney were taken after 30 days experiment. The urea levels were significantly higher in P1 (p=0.00) and P3 (p=0.01) in compare to KN, by 67% and 54% respectively. Creatinine levels were significantly lower in all watermelon intake groups by 32-46% in compare to KP. Kidney damages were obvious in group KP (p=0.00) and P1 (p=0.01). This research demonstrated that MSG exposure caused kidney damages and decreased kidney function. Watermelon juice at the concentration of 50% is able to reduce kidney damages by 48%.

Keywords: monosodium glutamate, kidney, watermelon juice, wistarrats

Abstracts
Monosodium glutamate (MSG) is a food additive used in processed food. Long time use of MSG causes oxidative stress and damages kidney. The lycopene content in watermelon is potential in reducing oxidative stress. This research aimed to discover the effect of watermelon juice in preventing kidney damage in rats. 30 Wistar rats were divided into 5 groups: four groups fed with 10 mg of MSG / g body weight; three groups of these also fed with 3 different concentrations of watermelon juice (25% in P1, 50% in P2, and 100% in P3) whereas the MSG only is as the control positive (KP). The fifth group is supplemented with water as the negative control (KN). Blood and kidney were taken after 30 days experiment. The urea levels were significantly higher in P1 (p=0.00) and P3 (p=0.01) in compare to KN, by 67% and 54% respectively. Creatinine levels were significantly lower in all watermelon intake groups by 32-46% in compare to KP. Kidney damages were obvious in group KP (p=0.00) and P1 (p=0.01). This research demonstrated that MSG exposure caused kidney damages and decreased kidney function. Watermelon juice at the concentration of 50% is able to reduce kidney damages by 48%.

Conflict of interest: No conflict of interest.
Kidney damage and decreased function due to MSG cause an increase in reactive oxygen species (ROS) that result in oxidative stress. MSG causes an increase in succinyl CoA ligase that increases α-ketoglutarate dehydrogenase (α-KGDH) activity and ROS production. An increase in α-ketoglutarate also occurs as MSG increases glyceraldehyde 3 phosphate dehydrogenases which causes catalysis of NADH-dependent superoxide. MSG increases intracellular Ca\(^{2+}\) via (N-methyl-D-aspartate)NMDA that activates nitrate synthase and protein kinase C. The activation of nitric oxide synthase and protein kinase C activates free radicals and lipid peroxidation that play a role in oxidative stress [14].

The increase in oxidative stress can be reduced by consuming fruits or vegetables that are rich in antioxidants. One compound in fruits and vegetables that functions as an antioxidant is lycopene. Lycopene is a phytochemical hydrocarbon of the carotenoid group which acts as an antioxidant and is conjugated in countering the lipid peroxidation radicals[15]. Lycopene is found in some fruits such as watermelons which contain 4.5 mg/100 g of fresh weight [16].

Watermelons (Citrullus lanatus) are one of widely preferred fruits for its taste and water content [17]. It is well known that watermelons are effective to reduce oxidative stress by phytochemical lycopene[18].

1. Methods

This is an experimental study with the post-test only control group design. The rats were fostered at the Department of Pharmacology. Histological preparation of samples and analysis of histopathological changes conducted at the Anatomical Pathology Laboratory. Serum creatinine and urea levels were analyzed at the Integrated Laboratory of the Faculty of Medicine. The research used 30 male Wistar rats (Rattus Norvegicus), 8 – 12 weeks old and body weight 150 - 200 g. The rats were divided into 5 groups, consisting of 3 treatment groups (P1, P2, and P3), 1 positive control group (KP) and 1 negative control group (KN), 6 rats each. Drop Out criteria were 1) rats become sick during the keeping period (observation period, 7 days before treatment); 2) sick or died during treatment; 3) sick or died before terminated for sampling.

1.1 Procedure

This research was approved by the Animal Research Ethics Committee of the Faculty of Mathematics and Natural Sciences/AREC FMIPA of University of Sumatera Utara (Registration number 0411/KEPH-FMIPA/2019). Before conducting the research, the rats were raised for one week, at natural temperatures and equipped with food and water. They were divided into 5 groups consisting of: negative control group (KN), positive control group (KP) given 10 mg/g BW of MSG, Treatment Group 1(P1) given 10 mg/g BW of MSG plus 25% concentration of watermelon juice, treatment group 2(P2) given 10 mg/g BW of MSG and 50% concentration of watermelon juice, treatment group 3(P3) given 10 mg/g BW of MSG plus 10% concentration of watermelon juice, orally for 30 days. 

**Watermelon Juice Making.** Watermelons were washed cleanly to remove dirt and insecticide residues, cut the red to white part, and weighed 15 kg to produce ± 13 L of watermelon juice (containing ±681.72 mg lycopene), by blending with a blender for 2 minutes (during the blending process, the blender is switched on intermittently to reduce heating to the blender). Then, the blended watermelons were filtered using a filter to separate the juice from the pulp. After that, the watermelon juice was stored in a freezer at temperature of -20° C in separate packages (each containing 450 ml, for daily administration). On the day of administration, the juice was firstly from the freezer ± 2 hours before administration. The dilution was done by pouring the juice into 2 cups of 20 ml each. The first glass as dose I had 100% concentration, the second glass was added with 20 ml of water as dose II had 50% concentration, and then 20 ml of the second glass was added with 20 ml of water as dose III with 25% concentration.

**Supplementation of MSG and Watermelon Juice.** MSG (10 mg/g BW) was given orally, watermelon juice was given by ad libitum feeding.

**Sampling.** Rats were sacrificed on the 31st day, after weighing. Surgeries were performed, then blood samples were taken with cardiac puncture. The blood was put into a vacutainer tube and centrifuged 6000 rpm for 15 minutes. Serum samples obtained were transferred to a 1.5 ml micro tube and stored at -20°C for further assay. Then, the kidney samples were taken and cleaned using 0.9% of NaCl.

**Preparations and Histological Examination.** Organ samples were put into 10% of formalin buffer. Then dehydration was done through a series of alcohol solutions, and impregnation was done next (paraffin infiltration) using liquid paraffin, and next was Embedding (paraffinization), sectioning (pemotongan), using a 5μm microtome, staining with Hematoxylin Eosin (H&E) and examining under a light microscope (Olympus CX21, with magnification of 40x and 400x).

Observations were made by dividing the preparations into 4 parts, the percentage of kidney damage area from the four parts was added up and divided by four. The extent of cellular damage to the glomerulus was indicated by hyper cellularity (caused by infiltration and proliferation of endothelial and mesangial cells), atrophy (indicated by Bowman’s dilatation and glomerular contraction, proximal tubules and distal tubules), and necrosis (indicated by degeneration of nucleus such as karyopyknotic, karyorrhexis, and karyolitic).
Assessment of the kidney damage level is made according to Santoso et al. (2006) as follows: Score 1 (Normal) if kidney damage is not found; Score 2 (Mild) if kidney damage area is < 25%; Score 3 (Moderate) if kidney damage area is 25-50%; and Score 4 (Severe) if kidney damage area is > 50%.

**Urea Level Assessment.** Serum urea levels were examined using a Urea kit (Glory Diagnostic and random). Urea mono-reagent was made by mixing reagent 1 and 2 with scale 1:2. Mono-reagent was left for 30 minutes. It must not be exposed to light. After the mono-reagent was stable, 1000 µl of mono-reagent was mixed with 10 µl of serum samples, serum urea levels were assessed using a UV spectrophotometer in the first and second minutes. Then the absorbance assessment results were input into the urea calculation formula, as follows:

\[
\text{Urea} = \frac{\Delta A_{\text{Samples}} \times \text{standard concentration (mg/dL)}}{\Delta A_{\text{Standard}}}
\]

**Notes:**
- \(\Delta A_{\text{Samples}} = A1-A2\)
- Standard Concentration = 50 mg/dl

**Serum Creatinine Levels Assessment.** Serum creatinine levels were assessed by creatinine kit (Glory Diagnostic and random). Creatinine mono-reagent was made by mixing reagent 1 and 2 with scale 4:1. Then, 1000 µl of mono-reagent was mixed with 50 µl serum samples; serum creatinine was assessed using a visible spectrophotometer in the first and third minutes. Then the absorbance assessment results were input into the creatinine calculation formula, as follows:

\[
\text{Urea} = \frac{\Delta A_{\text{Samples}} \times \text{standard concentration (mg/dL)}}{\Delta A_{\text{Standard}}}
\]

**Notes:**
- \(\Delta A_{\text{Samples}} = A2-A1\)
- Standard Concentration = 2 mg/dl

### 2.2 Data Analysis

The data among treatment groups were analyzed by using ANOVA testing. If a significant change is found, it is proceeded to Post Hoc testing with LSD analysis at rate of 5%, and tested by Mann Whitney testing to compare the results among the groups.

### 2. Results and Discussions

The research on the influence of watermelon juice on rats’ kidneys which were exposed to MSG discovered rats’ kidney functions and histopathological description.

#### 2.1 Kidney Function

Table 1 shows that the highest mean of serum urea is found in group P1 (8.02±2.26 µmol/l) whereas the highest mean of serum creatinine is found in group KP (63.97±12.63 µmol/l). The results of the statistical test found out that urea levels were found higher and different in groups KP (\(p=0.01\)), P1 (\(p=0.00\)) and P3 (\(p=0.02\)) compared to group KN. Creatinine levels were found lower and significantly different from control groups in groups P1 (\(p=0.00\)), P2 (\(p=0.04\)) and P3 (\(p=0.02\)).

**Table 1: Urea and Creatinine Levels**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Urea (µmol/l)</th>
<th>Serum Creatinine (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KN</td>
<td>4.81±0.68</td>
<td>59.43±6.05</td>
</tr>
<tr>
<td>KP</td>
<td>7.56±2.05*</td>
<td>63.97±12.63</td>
</tr>
<tr>
<td>P1</td>
<td>8.02±2.26*</td>
<td>34.33±11.75*</td>
</tr>
<tr>
<td>P2</td>
<td>5.58±1.68</td>
<td>43.21±6.29*</td>
</tr>
<tr>
<td>P3</td>
<td>7.41±1.52*</td>
<td>42.02±4.66*</td>
</tr>
</tbody>
</table>

Values: Mean ± SD. Negative control (KN), Positive control (KP); Treatments (P1, P2, P3). N=6. *Significant difference compared to positive control group (KP).

#### 2.2 Kidney Histopathology

Table 2 demonstrates that the normal kidney structure are mostly found in group KN (66.67%), the mild damages were mostly found in groups P1 and P3 (66.67%), the moderate damages were mostly found in group KP (50%) and the severe damages were mostly found in group KP (50%). The statistical testing results showed that the kidney damage scores were higher and significantly different in KP (\(p=0.00\)) and P1 (\(p=0.08\)) compared to KN.

**Table 2: The structural damage scores of rats’ kidneys**

<table>
<thead>
<tr>
<th>Group</th>
<th>Kidney damage level in number and percentage</th>
<th>TOTAL</th>
<th>Mean of Kidney Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Mild</td>
<td>moderate</td>
</tr>
<tr>
<td>KN</td>
<td>66.67</td>
<td>33.33</td>
<td>0</td>
</tr>
<tr>
<td>KP</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>P1</td>
<td>0</td>
<td>66.67</td>
<td>33.33</td>
</tr>
<tr>
<td>P2</td>
<td>33.33</td>
<td>50.00</td>
<td>16.67</td>
</tr>
<tr>
<td>P3</td>
<td>16.67</td>
<td>66.67</td>
<td>16.67</td>
</tr>
</tbody>
</table>

Values: Mean ± SD. N=6. Negative control (KN); Positive control (KP); Treatments (P1, P2, P3). N=6. *Significantly different from KN

Table 3 shows that the highest mean of Bowman’s capsule diameter is found in group P2 (60.59±6.70 µm) while the lowest one is found in group KP (55.07±5.84 µm), the largest diameter of glomerulus is found in group P2 (43.56±6.27 µm) and the smallest is found in group KP (38.78±5.50µm), the highest mean of the differences between Bowman’s capsule diameter and glomerulus is found in group P3 (0.758 µm). The statistical testing results found out that the mean of glomerulus diameter in group P1 is smaller and significantly different (0.035) than that of in group KN.
kidney tissue by inducing oxidant stress that changes the description of kidney histopathology. The research done by Singh et al, 2014, states that kidney structure becomes not regular, glomerulus undergoes vacuoles partial atrophy, nucleus pyknotic and dilatation of Bowman capsule. The display of kidney tubules is indicated by epithelial layer degeneration [11].

Meanwhile, the research by Elbassuaoni studying the effect of MSG on kidney function, discovered a description of photomicrographs, showing irregular kidney structure, glomerulus with vacuoles partial atrophy, nucleus pyknotic and dilatation of Bowman capsule. The display of kidney tubules is indicated by epithelial layer degeneration, in line with the research done by Minarma, on description of kidney histology and function after administration of MSG; the results demonstrated changes in the description of kidney histopathology i.e. edema in glomerulus and kidney tubular cells.

Damas in either glomerulus or tubules caused by imbalanced reactive oxygen stress (ROS) are resulted from MSG. The activities of N-methyl-D-aspartate (NMDA) receptor, metabotropic glutamate receptor (mGlur) and cysteine-glutamate antiporter are excessive, so that the glutamate levels increases and amino acid is filtrated freely by glomerulus which is followed by an increase in calcium in cytoplasm leading to disorders in Na\text{\textsuperscript{+}} ATPase channel [13]

The study done by Pinto 2001, found out that consumption of 150 ml of watermelon juice could produce antioxidant effects in humans’ blood [24]. Daily intake of 5 mg lycopene is effective to ve antioxidants compared to higher intake more than 20 until 30 mg per day, due to limited absorbance of lycopene [25]. According to Rao, the recommended daily intake of lycopene averages in 5-10 mg per day, in which watermelon can be consumed in form of juice or fresh fruit [26]. Lycopene functioning as antioxidants is also conjugated in repelling lipid peroxidation radicals, reactive oxygen species (ROS), and nitrate oxide [27].

Some researches demonstrate that lycopene had positive influences on humans’ health because of it various natures. It functions as antioxidant and has the most efficient capacity of oxygen single quenching and natural carotenoid (ten times more effective than \( \alpha \)-tocopherol). In addition, it also protects biomolecules from oxidative damages, impedes cellular proliferation, and modulates communication intracellular, particularly in stimulating gap junctional communication (GJC) [17]. The mechanism of lycopene in preventing oxidative stress in MSC (Cytoplasmic Membrane) is that oxidative stress triggers induction of reactive oxygen species (ROS) in MSC. ROS leads to phosphorylation of ATM and the signaling path related to apoptosis (p38 and JNK), resulting in augmentation of p53 phosphorylation. Furthermore, ROS

### Table 3: Bowman and glomerulus

<table>
<thead>
<tr>
<th>Group</th>
<th>Diameter of Bowman Capsule (µm)</th>
<th>Diameter of glomerulus (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KN</td>
<td>60.05±2.75</td>
<td>47.93±3.16</td>
</tr>
<tr>
<td>KP</td>
<td>55.07±5.84</td>
<td>39.55±5.35*</td>
</tr>
<tr>
<td>P1</td>
<td>55.08±7.41</td>
<td>36.94±4.21*</td>
</tr>
<tr>
<td>P2</td>
<td>60.59±6.70</td>
<td>45.23±7.08</td>
</tr>
<tr>
<td>P3</td>
<td>59.01±4.49</td>
<td>43.65±5.04</td>
</tr>
</tbody>
</table>

Mean ± SD. N=6 Negative control (KN); N=6 Positive control (KP); N=6 Treatment (P1, P2, P3). * Significantly different from KN.
breaks the balance between Bax and Bcl2, resulting in induction of apoptosis through division of PARP-1 and Caspase-3. Lycopene depresses apoptosis yang induced by oxidative stress through akt-MnSOD. Moreover, lycopene decreases phosphorylation of MAPK related to apoptosis (p38 and JNK). This effect of lycopene on oxidative stress induces a decrease in phosphorylation of ATM-p53 signaling path, and protection for PARP-1 and caspase-3 divisions, resulting in prevention of apoptosis and augmentation of life continuity in MSC. ATM; ataxia telangiectasia mutates serine / threonine protein kinase, MAPK; protein kinase is activated by mitogen, PARP-1; poly (ADP-ribose) polymerase 1, PI3K; phosphatidylinositol-4,5-bisphosphate 3-kinase [28].

3. Conclusion
The research results demonstrated that exposure to MSG can cause tissue damages and decreased kidney function. Watermelon juice 50% is able to repair oxidative stress which results in kidney damages.

Acknowledgments
The authors gratefully acknowledge that the present research is supported by University of Sumatera Utara.

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