Acute effect of tomato extract (*Lycopersicum esculentum*) on rat’s (*Rattus norvegicus*) behavior and body weight

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Abstract: Tomato is one of the natural beneficial fruits. It contains lycopene, one of the effective carotenoids, also tocopherol, Vitamin C, Vitamin A and many more nutrition. Mostly, people consumes tomato uncontrolled. It commonly consumed in large quantities and for long periods of time. Based on that cases, it needs to be considered because the consumption of excess antioxidants can trigger a back reaction and increase pro-oxidant compounds. Therefore, this research was to determine the safety level of pure tomato consumption. This research is an experimental research with high tomato dose as treatments. Five group i.e. control (K) 0 mg/ individual tomato extract; K1, 16 mg/ individual tomato extract; K2, 160 mg/ individual tomato extract; K3, 1600 mg/ individual tomato extract; and K4 16,000 mg/ individual tomato extract. Natural tomato extract doses that used for supplementation did not have acute toxicity. The observed behavior in rats did not show any significant difference between treatment and control group. This is probably the dose used in the study is still in tolerant dose for rats metabolism. So, it can be concluded that there is no acute effect on consumption of lycopene extract from tomato.

1. Introduction
Atherosclerosis is a cardiovascular disease caused by hyperlipidemia, and triggers coronary heart disease (CHD) and stroke [1]. Various studies have been proven that consumption of tomatoes can reduce total cholesterol levels [2] triglycerides and LDL cholesterol and increase HDL cholesterol levels [3]. This reduces the potential for atherosclerosis to become worse [4].

Tomato contains very powerful antioxidants such as lycopene, Vitamins C and E and carotenoids and Vitamin A. Every one gram of steamed tomato extract contains 46.92 µg of lycopene, 586.44 µg of β-carotene, 22.98 mg/ 100g of Vitamin C and 0.41 mg/ 100 g of α-tocopherol [5].

The problem faced is that consumption of tomatoes by the community is often uncontrolled. Tomatoes are usually consumed in large quantities and for long periods of time. This problem needs to be considered because the consumption of excess antioxidants can trigger back reaction and increase pro-oxidant compounds [6], especially in the liver and kidneys condition [7].

The use of traditional plants/ traditional medicines needs to be proven their safety and effectiveness through research. This is based on findings in the community regarding the use of traditional drugs that have negative effects and are toxic. Responding to these problems this study aims to establish the potential for acute toxicity by determining the LD50 value in atherosclerotic rat induced by tomato extract through physical observation and behavior.

2. Method
This research is an experimental research, posttest randomized controlled group design. The study was conducted at the Biochemistry Laboratory of the Department of Biology, Faculty of Mathematics
Science, Universitas Negeri Semarang and the Molecular Biology Laboratory, Faculty of Medicine, Universitas Gadjah Mada.

2.1. Tomato extraction
The total tomato extract used in this study was 500 mg/ day for 30 days or as many as 15 kg of tomato extract. To comply needed extract, as many as 50 kg fresh tomatoes were steamed for 30 minutes at temperature of 120 °C, then crushed with blender then extracted using maceration method with 70% alcohol. After maceration, the tomatoes were put into the oven at 40-50 °C until dry. After that, the extract were dried into a rough powder. The results of the blender were sieved with number 100 sieve, the fine powder obtained was weighed as much as 15 kg. The dissolution process was carried out using distilled water for two times [8].

2.2. Rat supplementation
The samples of this study were Sprague Dawley (SD) Rattus norvegicus rats which were kept in a cage and fed with 594 pellets (PT Japfa Confeed Indonesia: Grobogan, INDONESIA) on an ad libitum basis. A total of 25 healthy male SD rats, aged 12 weeks and body weight of around 200 - 300 grams, were divided into five groups. The first group (K) was a control which was given a solvent of tomato extract. The second to fifth group were treated in the form of tomato extract with a dose of 0, 16, 160, 1600 and 16000 mg/ head, respectively.

The extract was done once in a round. Rat were nurtured and observed for 24 hours to record rat mortality, weight, morphometry and morphology of rat; hair loss; aggressiveness; responsiveness and activity. Observation of activities and conditions of rats was carried out at 0, 30 and 1, 2, 4 and 24 minutes. The measurement results of the paramater were tested using One-way ANOVA and or chi square with a 95% confidence level, if significant effect continued with the test. continued, with a 95% confidence level. Statistical analysis was performed with Statistical Product and Service Solution (SPSS) 24 data processing facilities and Windows. Data is presented in the form of processed and interpreted data.

3. Result and Discussion
3.1. Body weight and urine production
The administration of tomato extract on R. norvegicus has a variable effect on body weight, urine production and neurobehavior. Significant differences occurred in the body weight of male rats induced with tomato extract of 1600 mg/ ind i.e. 197.60±16.98 gr in the P3 group with the control group 167.00±7.84 and P4 was 162.40±3.27 gr. Whereas in female rats, the largest weight of rats was in the P2 group given 160 mg / ind tomato extract, which was 171.00±21.04. This was significantly different from the control group, namely 144.00±19.24 gr and P3 group at 160.20±3.27 gr and the P4 group which was 141.40±34.93 gr. Although there were significant differences, in the group induced with tomato extract and control. In the P4 group there was no significant difference with the control group (Table 1). This indicates that the administration of tomato extract at high doses does not affect the change in body weight of rat for 24 hours.

| Group | Male (gr) | Female (gr) |
|-------|-----------|-------------|
| C     | 167.00±7.84<sup>ab</sup> | 144.00±19.24<sup>ac</sup> |
| P1    | 184.40±23.55<sup>bc</sup> | 189.60±18.41<sup>b</sup> |
| P2    | 196.60±7.64<sup>c</sup> | 171.00±21.04<sup>bc</sup> |
| P3    | 197.60±16.98<sup>c</sup> | 160.20±3.27<sup>a</sup> |
| P4    | 162.40±10.31<sup>a</sup> | 141.40±34.93<sup>a</sup> |

<sup>abc</sup> significantly different
The difference in body weight is probably caused by the method of giving tomato extract directly orally in P1-P3 group and indirectly for P4 group. The tomato extract were given to P4 group by mixed and formed as rat’s feed. The difference in treatment is based on the amount of tomato extract that must be given. Giving tomato extract through oral method or directly put into the stomach gives an opportunity for rats or time to eat more. Whereas in P4 group, tomato extract was made into feed, so that rats eat their food or nutrition intake was only from tomatoes. The increase in eating is also related to the amount of drinking rat which then affects urine production [9].

Table 2. Average of total rat’s urine production after 24 hours tomato extract supplementation

| Treatment | Male        | Female*       |
|-----------|-------------|---------------|
| C         | 4.33 ± 0.11b | 0.90 ± 0.05   |
| P1        | 2.38 ± 0.17b | 0.96 ± 0.05   |
| P2        | 2.91 ± 0.11b | 0.82 ± 0.05   |
| P3        | 1.46 ± 0.16b | 0.65 ± 0.08   |
| P4        | 0.27 ± 0.89b | 0.62 ± 0.08   |

*Significantly different, * means there are no different

The main consumption of disonde rats was pellets containing higher carbohydrates, lipids and proteins than tomato extracts consumed by rats in the P4 group. Pellet consumption resulted in water consumption by rats in group C, P1-P3 was higher than P4. This was shown in the urine production of male rats which were significantly different between control rats and urine production treatment (Table 2). Control rat produced a total urine of 4.33 ± 0.11 ml which was much higher than that of P4 rat which was 0.27 ± 0.89. Hourly urine expenditure also showed an increase, but the urine per hour between groups was not significantly different. Urinary excretion actually experienced a significant increase in male rats in the control group, in the last six hours (Figure 1).

Figure 1. Male rat urine production per hours after tomato extract supplementation

Whereas in female rats, urine production in the group given tomato extract did not have a significant difference (Table 2). Hourly urinary excretion in female rats also did not show fluctuating results (Figure 2) This indicated that tomato extract had no direct effect on urine production which indicated no interference with urinary organs.
3.2 Motor activity and neurobehaviour after tomato extract supplementation

Based on observations of movement and movement of rat, there were significant differences between P3 groups with other groups in male rats, and P1 groups with other groups in female rats. However, based on the LSD analysis in the rat group, showed that there was no significant difference between the control groups, namely the displacement of 6.8 ± 0.84 cm with a speed of 0.44 ± 0.06 cm / s compared to the P4 group which moved as far as 7.4 ± 0.55 cm with a speed of 0.45 ± 0.036.

The same thing happened to female rats where there were no differences in control rats with P3 and P4 rats. In fact, the P3 and P4 groups were rats supplemented with high-dose tomato extract. In the control female rat group, the displacement occurred as far as 7.6 ± 0.36 cm with a speed of 0.43 ± 0.03 cm / s, whereas in the P3 group, the rat moved as far as 7.2 ± 0.55 cm with a speed of 0.44 ± 0.043 cm / s and the P4 group moved 7.4 ± 0.45 with a speed of 0.40 ± 0.049 cm / s (Table 3).

This indicates that the motoric activity of rat was not disturbed by the provision of tomato extracts, or in other words high-dose tomato extracts were given no toxic effect on SD rat. The dose of tomato extract reaches 1000x optimal dose of 16 mg / Ind [4], still in the tolerant phase or safe dose for SD rat.

Table 3. Results of motor activity assessment for Sprague Dawley rats fed tomato extract at up to 16000 mg/ Ind for 24 hours

| Endpoint                  | K           | P1          | P2           | P3           | P4           |
|---------------------------|-------------|-------------|--------------|--------------|--------------|
| **Female**                |             |             |              |              |              |
| Total distance moved (cm) | 7.6±0.36    | 6.6±0.47    | 6.8±0.45     | 7.2±0.55     | 7.4±0.45     |
| Time of movement (s)      | 17.50       | 13.32       | 17.70        | 16.28        | 18.40        |
| Mean velocity (cm/s)      | 0.43±0.03   | 0.49±0.037  | 0.38±0.03c   | 0.44±0.043a  | 0.40±0.049ac |
| **Male**                  |             |             |              |              |              |
| Total distance moved (cm) | 6.8±0.84    | 7.2 ±0.84   | 7.4 ±0.55    | 7.6 ±0.55    | 7.4 ±0.55    |
| Time of movement (s)      | 15.5        | 12.6        | 18.26        | 13.16        | 16.28        |
| Mean velocity (cm/s)      | 0.44±0.06a  | 0.57±0.06a  | 0.41±0.031a  | 0.58±0.27b   | 0.45±0.036a  |

Other observations related to neurobehaviour also showed no symptoms of poisoning. Observation of neurobehavior which includes activities as mentioned in table 4. Each mouse shows hanging activity, the same touch and piercing response. As for the tail puncture reflex response and flashing...
reflex both show no difference between groups. Common symptoms that appear from poisoning conditions due to exposure to active compounds are, decreased reflexes, changes in behavior and changes in environmental responses [10]. However, this condition does not appear in observing mice.

Some conditions that show differences are tremor conditions in the female P4 group and male P3 and P4. In addition, differences in the condition of the piloerection (standing hair) were shown in the male P4 and female P4 groups. But the difference in conditions is not followed by differences in conditions and or other activities.

Table 4. Functional observational battery results for SD rats fed low to high dose of tomato extract for 24 hours

| Criteria                      | K     | P1    | P2    | P3    | P4    |
|-------------------------------|-------|-------|-------|-------|-------|
| **Female**                    |       |       |       |       |       |
| Hanging                       | 5.0±0.4 | 5.0±0.4 | 5.0±0.4 | 5.0±0.4 | 5.0±0.4 |
| (score 1-5)                   |       |       |       |       |       |
| Touch response                | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 |
| (score 1-5)                   |       |       |       |       |       |
| Tail pinch response           | 5.0±0.1 | 5.0±0.1 | 5.0±0.1 | 5.0±0.1 | 5.0±0.1 |
| (score 1-5)                   |       |       |       |       |       |
| Palpebral closure             | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 |
| (score 1-5)                   |       |       |       |       |       |
| Tremor                        | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 2.0±0.5 |
| (score 1-5)                   |       |       |       |       |       |
| Salivation                    | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| (score 1-5)                   |       |       |       |       |       |
| Lacrimation                   | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| (score 1-5)                   |       |       |       |       |       |
| Diarrhea                      | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| (score 1-4)                   |       |       |       |       |       |
| Piloerection                  | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 2.0±0.8 |
| (score 1-4)                   |       |       |       |       |       |
| **Male**                      |       |       |       |       |       |
| Hanging                       | 5.0±0.4 | 5.0±0.4 | 5.0±0.4 | 5.0±0.4 | 5.0±0.4 |
| (score 1-5)                   |       |       |       |       |       |
| Touch response                | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 |
| (score 1-5)                   |       |       |       |       |       |
| Tail pinch response           | 5.0±0.1 | 5.0±0.1 | 5.0±0.1 | 5.0±0.1 | 5.0±0.1 |
| (score 1-5)                   |       |       |       |       |       |
| Palpebral closure             | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 | 5.0±0.0 |
| (score 1-5)                   |       |       |       |       |       |
| Tremor                        | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 5.0±0.4 | 3.0±0.5 |
| (score 1-5)                   |       |       |       |       |       |
| Salivation                    | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| (score 1-5)                   |       |       |       |       |       |
| Lacrimation                   | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| (score 1-5)                   |       |       |       |       |       |
| Diarrhea                      | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| (score 1-5)                   |       |       |       |       |       |
| Piloerection                  | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 5.0±0.1 |
| (score 1-4)                   |       |       |       |       |       |

Physically, motorically and neurobehavior, the condition of rats supplemented with tomato extract up to 16000 mg / Ind showed no significant effect, but differences in conditions in some aspects such
as tremor and selection need special attention. Based on the analysis in this study, the difference in conditions is still a question. Therefore, further research is needed to determine the cellular, tissue and physiological conditions of the administration of the high-dose tomato extract. Physiological parameters at the cellular and tissue levels can be used as the main basis in determining of the toxicity level [11].

4. Conclusion
Administration of tomato extract up to 16000 mg / individual on Rattus norvegicus rats Sprague dawley strain showed no symptoms of poisoning. The results showed no significant changes in motoric activity and neurobehavior.

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