

## Antioxidant activity of tomato juice rich in lycopene antioxidant as degenerative chemopreventive agents using *citrus aurantifolia* juice as a preservative

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**Abstract.** The fruit of tomato (*Solanum lycopersicum Lycopersicum esculentum SYN.*) plant from Solanaceae family that originated, has a content of vitamins A and C as well as antioxidant compounds that are good for health especially lycopene. Lycopene is a carotenoid compounds found in red yellowish vegetables and fruits. The chemo preventive agents that have been scrutinized was antioxidant-rich tomato juice using lime juice as preservative, applied on rats which had been induced by carcinogens i.e Acrylamide compounds that can cause cancer. Analysis of antioxidant activity is done by observing the effect of lycopene antioxidant-rich tomato juice on rat liver histopathology using hematoxylin eosin staining method and influence of the Cu-Zn SOD enzyme (Copper Zink-superoxide Dismutase) using immunohistochemistry staining. At 503 nm wavelength obtained Lycopene antioxidant content was of average of 64,2582 mg/Kg with an incubation time of 30 minutes. Lycopene levels before pasteurization was 74,8454 mg/Kg which was higher than the levels of fresh plum tomatoes lycopene at 64,2582 mg/Kg with the incubation time (at the extraction process) of 30 minutes each. The best lycopene level was found in the best storage of 2 weeks which was equal to 85,2148 mg/Kg at the preservatives concentration of lemon juice of 1%. There were some damage found in the liver cells of positive control group (K+), the first treatment group (P1) and the second treatment group (P2) where each group was given a diet of Acrylamide as a carcinogen compound of 2,0740 mg per day for 11 days.

**Keywords:** *Lycopene, Tomato, Antioxidant activity, preservative, Acrilamide.*

### 1. Introduction

Tomato fruit (*Solanum lycopersicum syn. Lycopersicum esculentum*) is a plant that comes from the *Solanaceae* family, has vitamin A and C content and lycopene compounds that are good for health. Overall the content of tomatoes per 100 grams is 30 kilo calories, 40 mg vitamin C, vitamin A 1500 SI, a number of iron, calcium, magnesium, potassium, iodine, zinc, fluoride, and organic acids. The benefits of consuming tomato juice regularly can reduce the risk of cancer, especially prostate cancer and can reduce the risk of heart disease and make the heart and blood vessels stronger. The other benefits of tomato juice can stimulate the body's metabolism, make the immune system stronger, in addition to being an antioxidant that is good for the body. The antioxidant activity of the tomato fruit extracts in methanol have IC50 values for menonantifikan that the antioxidant power of free radicals by 50% amounting to 44.06 ug / ml vitamin C lower than 3.63 ug / ml [1-4].

Lycopene is a carotenoid compound found in vegetables and fruits yellowish red. can be a chemopreventive agent for potential cancer. In a review article entitled *Lycopene in Oral Health* made in 2013 it was stated that lycopene showed a higher ability in single oxygen binding. Red tomato pigments contain *lycopene*, an antioxidant that can destroy free radicals in the body due to



smoking, pollution and ultraviolet light. In addition, *lycopene* is reported to prevent cell damage which can lead to cancer of the cervix, prostate, colon and pancreas. Lycopene has multiple functions in the body to protect the body from the beginning of cancer of the mouth such as leukoplakia and also prevent periodontal tissue destruction [1,5-6].

Various research activities on cancer in general is achieved by using an object experiment known as an animal model. In general, the animal model used for the study of cancer is a mouse. The studied chemopreventive agent was applied to mice that had been induced by carcinogens in *Acrylamide* compounds which can cause cancer. Mice will be sacrificed to analyze the antioxidant activity to prevent cancer by analyzing the levels of SOD ( *Superoxide Dismutase* ). SOD is a group of metalloenzymes that act as vital antioxidants and carry out primary protection against the toxic effects of superoxide radicals on aerobic organisms. SOD activity in all organs decreased significantly after lycopene was applied. [7-9].

The study entitled Antioxidant activity Fruit Tomato Rich in *Lycopene* as chemopreventive agent using citrus aurantifolia juice as a natural preservative is done in two phases for the purpose of Determining the antioxidant activity of fruit juice rich tomato *Lycopene* by DPPH method, Determine the levels of SOD in white rat liver induced by *acrylamide* at various concentrations of *Lycopene*- rich tomato juice [10-12].

Among the various antioxidants that have been found, lycopene is the most potent antioxidant in the sequence: lycopene > tocopherol > carotene > cryptoxanthine > zeaxanthin > carotene > lutein. Lycopene is an antioxidant by protecting cells from damage to singlet oxygen oxidation (oxygen quenching singlet) and other oxidizing agents. Singlet oxygen is a highly reactive oxygen molecule because it is at a high energy level. [13-14].

## 2. Materials and methods

Implementation the study was conducted from June to December 2014 in the Laboratory of Food Science, Faculty of Agriculture and Faculty of Science, Universitas Sumatera Utara Medan. material used in the study are: Lime (*Citrus aurantifolia*), Tomato, aqua mineral, NaOH, fenofalein indicators, reagents *phosphomolybdenum*, solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) (SIGMA-ALDRICH), Solvent methanol (MERCK), 1% starch indicator, iodine solution 0.01 N, Aquadest, Alcohol 70%, K Apas, Label, Medium Nutrient Agar (NA), and tissue. The instrument used is a spectrophotometer. Tomato juice was stored and analyzed for Lycopene levels, antioxidant activity in vitro with DPPH at each storage period.

Research using completely randomized design (CRD) with two factor treatments

A: The addition of lemon juice as a preservative: addition of lime juice preservative 0%, 0, 5%, 1%, Factor B : Storage of tomato juice in plastic cups: 0 days, 5 days, 10 days, and 15 days. Repeat, r will be done 3 times. Analysis carried out through Variance Analysis (ANOVA) based on the F-Test.

### **Determination of antioxidant levels of Lycopene by spectrophotometric method**

Fresh tomatoes are mashed with a blender then weighed 5 grams, put in a covered Erlenmeyer coated with aluminum foil on the outside and protected from light add 50 ml of solution (hexane: acetone: ethanol = 2: 1: 1) v / v, shaken for 30 minutes with a magnetic stirrer, transfer to a separating funnel then add 10 ml of distilled water then shake again for 15 minutes. Separate the polar layer and the non-polar layer, take all the top (non-polar) layers in a 100 ml volumetric flask, add organic solvents to the boundary markers. The total lycopene content was determined from the non-polar layer (the top) with UV-Vis spectrophotometer at a maximum wavelength of 471 nm [15].

### **Test of antioxidant activity with DPPH free radical scavenging assay**

#### Making DPPH solution

50 mg DPPH crystals are put into a 100 mL measuring flask, ethanol is added so the concentration becomes 0.05%. Then the dilution was carried out to obtain DP4 0.004% solution.

#### Test for antioxidant activity

Tomato juice was weighed as much as 0.25 g then added 1 mL DPPH. Ethanol is added so that the final volume is exactly 5 mL. The mixture is allowed to stand for 30 minutes. Homogenization is carried out for 20 seconds. Absorption read on a spectrophotometer at  $\lambda = 580$  nm. Solution form for tomato juice in ethanol. The control solution is DPPH without the addition of test solution.

Information :

Uptake Control: Uptake of DPPH radical at which the wave 580 nm. Sample

Uptake : Samples uptake in DPPH radicals at a wavelength of 580 nm (Desmiaty *et al.* , 2008)

### **Determination of Lycopene antioxidants in mice in vivo**

Twenty-five the Albino Sprague Dawley rat which weighed 160 - 200 grams , was adapted in the laboratory for 11 days and fed pellets and drank *ad libitum* . Then divided into 5 (five) groups - each consists of five (5) mice and treated as follows:

K0 : Normal Control (without giving Acrylamide , juice, only water)

K - : Negative Control (without giving Acrylamide , only tomato juice )

K + : Positive control was treated Acrylamide (without tomato juice)

P 1: Group treated Acrylamide and 2 ml of tomato juice per day

P 2: The group was given Acrylamide and 10 ml per tomato juice day

### Immunohistochemical staining procedure

Slide deparafinization using Xylol 1,2, and 3 solution for 5 minutes, then rehydration process was carried out to remove the remaining xylol using absolute alcohol solution, 96% alcohol, 80% alcohol, and 70% normal alcohol respectively 4 minutes, then washed under running water for 5 minutes. Enter the slide into the drying machine brand PT. Link Dako Epitope Retrieval, then set the *preheating* temperature 65°C, running time 98°C for 15 minutes, wait until  $\pm 1$  hour, after the slide is dry, using a pap pen marker, the preparations on the slide are circled so that when the antibody is penetrated or the other liquid does not cross the border of the pap pen circle. Immediately insert the slide into Tris Buffered Saline (TBS) pH 7.4 for 5 minutes, then block with peroxidase, for 5-10 minutes, then wash again in Tris Buffered Saline (TBS) pH 7.4 for 5 minutes, then blocking with 3% Normal horse serum (NSH), for 15 minutes, wash in Tris Buffered Saline (TBS) pH 7.4 for 5 minutes. After that it was incubated using SOD-1 polyclonal antibody (3458-100, PT. Bio Vision, 1:50 dilution) for 1 hour each. Wash again into Tris Buffered Saline (TBS) pH 7.4 or Tween 20 solution for 20 minutes, then drip with secondary antibody Dako Real Envision Rabbit / Mouse brand for 30 minutes. After that the slide is again washed in a solution of Tris Buffered Saline (TBS) pH 7.4 or Tween 20 for 5-10 minutes. Then slide drip with DAB + Chromogen Substrate Solution with 20 $\mu$ L dilution of DAB: 1000  $\mu$ L of substrate (hold 5 days at 2-8°C after mixing) for 5 minutes, wash again with running water and counterstain with Hematoxylin for 10 minutes each. Wash under running water for 5 minutes, dip slide prep a rat into lithium carbonate (5% in aqua) for 2 minutes, wash with running water and drying process fluid redone previously with the dehydration process using alcohol. This immunohistochemical staining test was done in Anatomy Pathology Laboratory, USU Faculty of Medicine.

### 3. Results and discussion

#### Characteristics of raw materials and tomato juice

The results of the test characteristics of the raw material of lime juice, tomato fruit, tomato juice before and after pasteurization can be seen in Table 1.

**Table 1.** Characteristics of lime juice, tomatoes, and tomato juice before pasteurization

NO	Raw material	Citric Acid (%)	Vitamin C (mg / 100g)	Lycopene (mg / Kg)	Activity Antioxidants(%)
1	Lime juice	7,376	293,0256	-	-
2.	Tomato	-	27,6406	64,2582	-
3.	Tomato juice before Pasteurization	-	-	74,8454	34,2746
4	Tomato juice after Pasteurization	-	127,0458	61,4884	44,7245

Antioxidant activity increased after pasteurization increased from 34.2746% to 44.7245% due to the application of heat in pasteurization causing other antioxidants besides lycopene which is bound to tomato fruit cells, namely the fruit flesh tissue in the form of fiber becomes released because the fibers soften.

### Lycopene levels offresh tomatoes

The test results of antioxidant levels of tomato Lycopene were carried out using spectrophotometric methods. Tomato fruit is blended and weighed to dissolve in organic solvents which will extract the Lycopene antioxidant during dark incubation. *Lycopene* antioxidant levels in fresh tomatoes are presented in Table 2 . At a wavelength of 503 nm, the average *Lycopene* antioxidant content was 64.2582 mg / Kg with an incubation time of 30 minutes. *Lycopene* content of tomatoes was tested by Onuoha *et al.* (2014) was 42.5 mg / kg and 53 mg / kg at 30 minutes incubation, and 88 mg / kg at 24 hours incubation. This difference is due to differences in growth, light intensity, maturity level, and incubation time during extraction. Increased levels of lycopene in incubation for 24 hours is relatively higher than 30 minutes, presumably the contact time between the ingredients with solvent is longer, lycopene in the cell will be extracted better.

**Table 2.** The antioxidant content of *lycopene* in fresh tomatoes

Tomato (Deuteronomy)	Mass (g)	incubation time	Abs. (503 nm)	Cons. <i>Lycopene</i> (mg / Kg)	Cons. <i>Lycopene</i> average (mg / Kg)
Tomato 1	0.09811	24 hours	0.718	100.5537	86.6891
	0.09811		0.52	72.8244	
Tomato 2	0.1500	30 minutes	0.737	67.5092	60.5476
	0.1500		0.585	53.5860	
Tomato 3	0.1503	30 minutes	0.7	63.9920	67.9687
	0.1503		0.787	71.9453	

### Lycopene levels of tomato juicebefore pasteurization

Lycopene content of tomato juice before the paste paste process can be seen in Table 3 , the incubation time is 30 minutes.

**Table 3.** *Lycopene* levels on tomato juice before pasteurization

Sari Tomato	Mass (g)	Absorbance(503 nm)	Konsentrasi <i>Lycopene</i> (mg / Kg)
1	0.1366	0.651	65.4813
2	0.1104	0.661	82.2658
3	0.1206	0.674	76.7891
		Average:	74.8454

Lycopene levels before pasteurization (74.8454 mg / Kg) were higher than the levels of lycopene fresh tomatoes (64.2582 mg / Kg) with an incubation time of 30 minutes each. Before pasteurization, fresh tomatoes have received heat treatment during the blanching process by steaming around 100°C for 10 minutes. During the heating process there are factors that influence lycopene content such as all-trans degradation and lycopene cis isomer , all-trans isomerization reaction to cycloisomer , and increasingly efficient tomato extraction process. The process of heating or high temperature is needed to disturb the cell so that all or most of the lycopene is released from the cell matrix [16].

### Antioxidant activity of *Lycopene*-rich tomato juice before pasteurization

Activity antioxidant of tomato juice after blanching, grinding, filtration, and homogenization process was determined by measuring the% value of antioxidant activity using DPPH method using a spectrophotometer. Tomato juice weighed as much as 0.3023 g

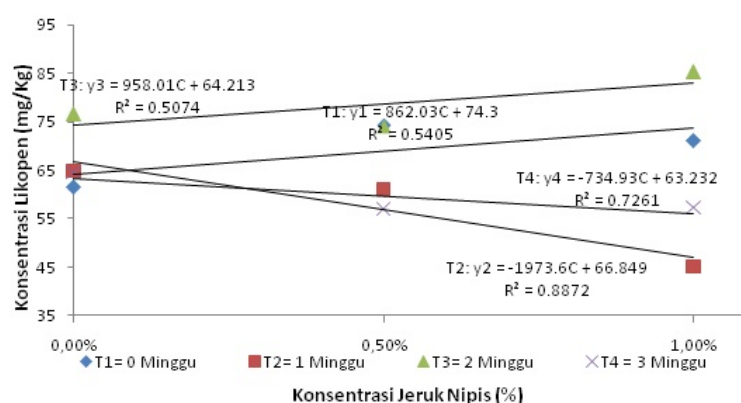
and then added 1 mL DPPH solution, dissolved with ethanol to a volume of exactly 5 mL.

The absorption value of tomato juice at  $\lambda = 580$  nm is 0.462 while DPPH solution is 0.789.

The antioxidant activity obtained was 34.2746%.

#### **Lycopene content of antioxidant Omatjuiceduring storage at various concentrations of lime**

Lycopene antioxidant levels were determined in spectrophotometry with  $\lambda = 503$  nm. The results of measurements of lycopene antioxidant levels are presented in Figure 1. The results of the observation showed that the higher the concentration of lime juice the levels of lycopene increased and the longer the storage levels of lycopene antioxidants decreased (Table 4). The significance value of statistical analysis was  $0.057 > 5\%$  which means that the treatment of adding lime juice and storage time did not significantly affect lycopene levels. Lycopene levels are best found at 2 weeks storage (T3). Storage of 3 weeks at each concentration of lime juice is not able to maintain lycopene levels of tomato juice.



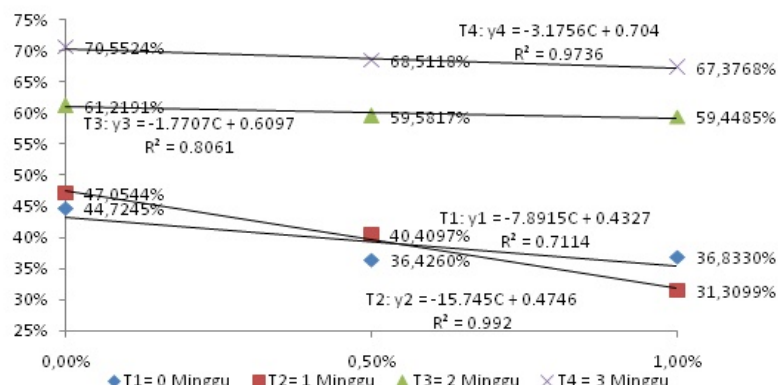
**Figure 1.** Relationship between lime juice concentration during storage against levels of antioxidant lycopene processed tomato juice

**Table 4.** Lycopene antioxidant levels of tomato juice at various concentrations lime during storage

Concentration of lime juice	Storage time			
	0 week	1 week	2 weeks	3 weeks
C1 = 0%	61.4884	64.8175	76.5944	64.5354
C2 = 0.5%	74.4534	61.0447	74.0210	56.9518
C3 = 1%	71.0684	45.0813	85.2148	57.1861

#### **Activities antioxidant juice tomato at various concentrations of lime juice during storage**

The antioxidant activity of tomato juice at various concentrations of lime juice during storage can be seen in Figure 2. From the observation of tomato juice during 3 weeks of storage at various concentrations of lime juice had a significant effect ( $p < 0.05$ ) can be seen in Table 5. Increased antioxidant activity can occur due to the spontaneous degradation of antioxidants in tomato juice and also because of the presence of bacteria.



**Figure 2.** Relationship value of antioxidant activity tomato juice for storage of the concentration of lime juice

Addition of citric acid causes a decrease in pH in tomato juice so that pathogenic bacteria that live in conditions of  $\text{pH} > 4.5$  will die. Decomposition bacteria can still survive conditions of pH 3.5 to 4.5. Acidic conditions will damage the bacterial cell membrane. Bacteria will produce degradation enzymes (amilase), organic compounds (in the form of poisons that will produce free radicals). Chemical compounds will damage membranes and DNA. Fat-soluble antioxidants can reduce lipid free radicals faster than oxygen and act as free radical catchers in the lipid chain peroxidation stage. The value of antioxidant activity decreases with increasing concentration of lime juice. The higher the concentration of lime juice, the amount of antioxidants in fruit juice derived from lime juice such as ascorbic acid and flavonoids, in addition to the antioxidant level of the tomatoes will decrease along with the increase in the amount of citric acid in processed fruit juice, so the value of antioxidant activity (IC50) will decrease.

**Table 5 .** Anova values various antioxidant activities of tomato juice concentration of citrus juice during storage

	Sum Squares	Of	F f	Mean square	F	Sig.
Between Groups	6116,802		11	556,073	12,479	.000
Within Groups	1069,422		24	44,559		
Total	7186,224		35			

### Observation of Cu Enzyme.Zn-SOD with Immunohistochemical Staining

The results of the observation of immunohistochemical appearance in the liver of rats given *acrylamide* diet and tomato juice rich in lycopene can be seen in Figure 3 . To overcome the danger that the team caused due to free radicals, the body developed a protective mechanism, namely the endogenous antioxidant consisting of enzymes and various compounds synthesized by the body. *Copper Zink Superoxide Dismutase* (SOD) enzyme is endogenous antioxidants that can be found on various body tissues. Superoxide dismutase is an enzyme which catalyzes dismutation of superoxide radical ions ( $\text{O}_2^-$ ) to hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and oxygen molecules  $\text{O}_2$  . *Copper zinc superoxide dismutase* (Cu, Zn-SOD) generally terd apat in the cytoplasm of eukaryotes are found in the extracellular fluids in mammals. Pe ngamatan Cu Zn SOD antioxidant content with immunohistochemical staining showed that the stronger the immunohistochemical appearance Cu Zn SOD namely the brown color of the lobular tissue of the hypocytes , the more content of Cu Zn SOD in the tissue [17-18,28,33].



Liver lobules in the group of negative control rats, rats fed with lycopene-rich tomatoes ( 3D images ) had a very dominant dark brown color compared to the group of neutral control rats that were not given either tomato juice or *acrylamide* carcinogens. Dark brown color showed that the SOD enzyme in rat liver cells the negative control group is positively strong. Exogenous antioxidants of tomato lycopene take over the endogenous antioxidant function of SOD as a leading line of defense in neutralizing and accelerating degradation free radical compounds to prevent damage to macromolecular components of cells . The supply of SOD endogenous enzymes became more prevalent in groups of mice given tomato juice compared to groups that were not given tomato juice, indicated by the intense brown color of SOD in the rat hepatocyte tissue. [30,34]

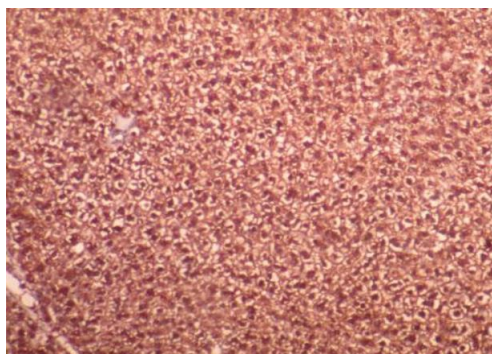


Figure3A. Neutral mouse liver cells (K0).Magnification of 100x

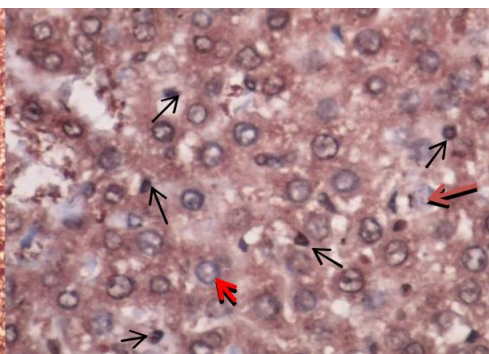


Figure3B. neutral mouse liver cells (K0).400x magnification.Full dark brown cells (black arrows) indicate strong positive SOD, light brown (brown arrows) indicating moderate positive SOD.The full brown and blue bar color cells (red arrow) indicate a weak positive SOD.

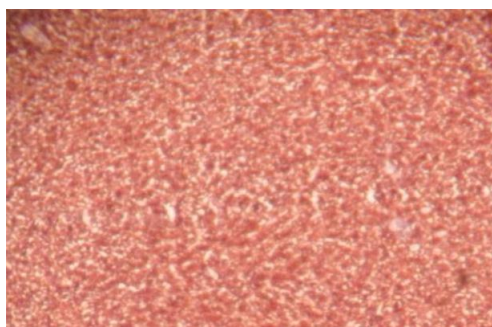


Figure3C. Rat liver cells negative control (K-).Magnification of 100x

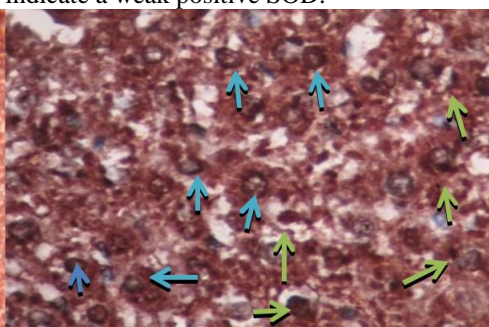


Figure3D. Rat liver cells negative control (K-).400x magnification.The dark brown color of hepatocytes is very dominant indicating the enzyme Cu. Zn SOD is a strong category.



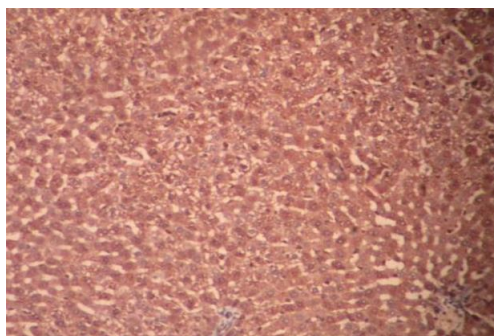


Figure3E. Positive mouse liver cells (K+).Magnification of 100x

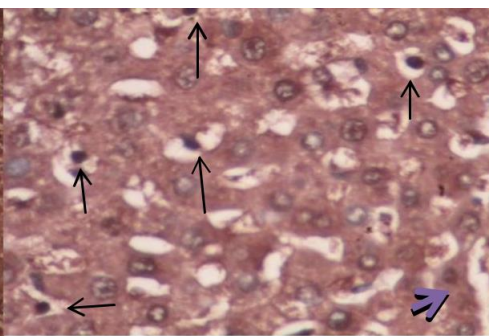


Figure3F. Positive mouse liver cells (K+).400x magnification.Selberwarnafulldark brownindicates a strong positive SOD.The white tissue partwidened,indicating no SOD.

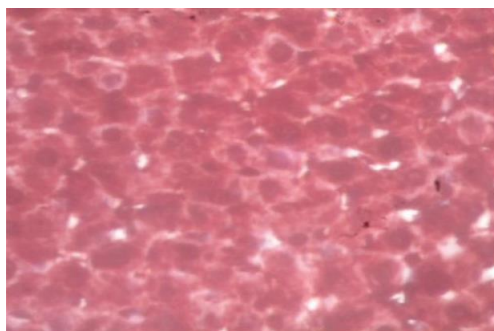


Figure3G. Rat liver cells The first treatment (P1).Magnification of 100x

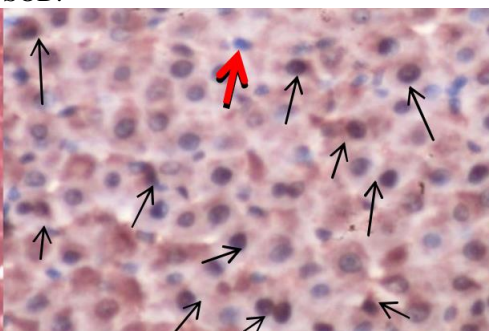


Figure3H. Rat liver cells First treatment (P1).400x magnification.A black arrow indicates a dark brown cell indicating a strong positive SOD, a red arrow indicating a blue intact cell indicating a negative SOD.

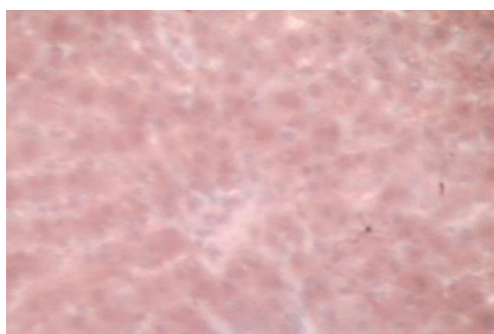


Figure3I. Rat liver cells Second treatment (P2).Magnification of 100x

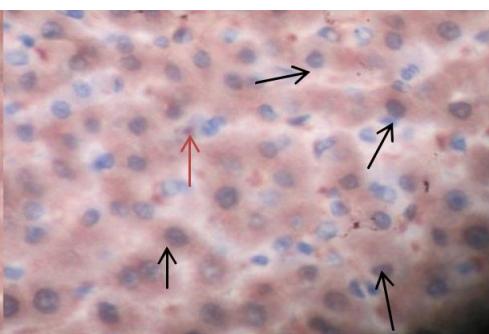


Figure3J. Rat liver cells Second treatment (P2).400x magnification.Only 4 whole cells with light brown color (black arrow) showed moderate positive SOD.A full brown light cell (red arrow) indicates a weak positive SOD.

**Figure 3 .** Photomicrograph localization Cu.Zn-SOD immunohistokimia rat liver tissue.

The K + mouse group had fewer numbers of full dark brown cells than the K0, K-, and P1 groups indicating that the mouse Hepatocyte SOD enzyme given carcinogens (Figure 3F) was the weakest compared to the group of mice that were not given *Acrylamide* carcinogens and rich tomato juice lycopene (Figure 3B, 3D, and 3H). Hepatocyte cells from the second treatment group (P2) had a positive SOD enzyme weaker than the first treatment group (P1). Dark brown color in P1 was more, while the blue color in P2 increased (Figure 3H and 3J). Oxidative stress in the P2 rat group was higher because this group was carried out 2 times of feeding of tomato juice every day for 11 days, while the P1 mouse group was only 1 time of strangulation.

#### 4. Conclusion

The best lycopene content of tomato juice obtained during storage for 2 weeks with lycopene concentration was 85.2148 mg / Kg for tomato juice with 1% lime juice preservative. *Acrylamide* compounds can cause damage to rat liver tissue. Exogenous antioxidants of tomato lycopene take over the function of the endogenous antioxidant SOD as the leading defense line in neutralizing and accelerating the degradation of free radical compounds to prevent damage to macromolecular components of cells. The supply of SOD endogenous enzymes became more prevalent in groups of mice given tomato juice compared to groups that were not given tomato juice, indicated by the intense brown color of SOD in the rat hepatocyte tissue.

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