

Efficacy of Lycopene Extracted from Tomato on Liver Enzymes and Tissues of Animal Model Infected with Acrylamide

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Lycopene is a carotenoid derivative, which is a natural pigment synthesized by plants and microorganisms during the photosynthesis process. The study aimed to extract lycopene from tomatoes using organic solvents in a ratio of 2:1:1 of hexane, acetone, and ethanol. The amount of lycopene extracted was (4.87 mg/100 g). We notice a decrease in the values of the liver enzymes ALT, ALK, and AST upon oral administration of lycopene to male infected rats compared to with their values when acrylamide is given alone. A change was also noted in the liver tissue after treatment with lycopene compared to the infected group, as there was an improvement in the central vein, lobules, hepatic sinusoids, and Coover cells after they were congested, expanded, and contained bloody bleeding. In conclusion, lycopene as an antioxidant phyto-component, can protect liver against damages caused by acrylamide (or acrylic amide). Tomatoes can be considering as functional vegetable for protecting liver from damages of chemicals such as acrylamide.

Keywords: Acrylamide, lycopene extraction, physiological properties, phytocomponent.

INTRODUCTION

Lycopene is a carotenoid derivative, which is a natural pigment synthesized by plants and microorganisms during the photosynthesis process to protect them from photosynthetic activity and increase photosensitivity (Rao *et al.*, 2003), and lycopene imparts the red color of vegetables and fruits rich in it (Shi and Maguer, 2000). Many studies, including (Rao and Ali, 2007), have indicated that eating tomatoes and their manufactured products containing lycopene contributes to the prevention of some chronic diseases due to its antioxidant activity, which is twice the activity of the rest of the beta-carotene components and ten times the tocopherol (Rao and Ali, 2007). Eating lycopene has also been linked to reducing the incidence of osteoporosis and decreasing bone density (Maggio *et al.*, 2006). Many recent studies have proven the ability of this chemical to prevent, protect, or reduce the harmful effects of free radicals, reduce oxidative stress in vivo, and reduce the incidence of many diseases., whether in humans or animals (Osazee *et al.*, 2024). Lycopene is the carotenoid responsible for the red color in tomatoes and their products. Helyes *et al.* (2009) indicated that tomatoes and their products represent the main sources of lycopene. Lycopene is a non-cyclic carotenoid that contains 13 double bonds. It is found naturally in every trans (trans) and is the

most stable form to heat treatments, and represents about 79-91% of the lycopene found in tomatoes (Kessy *et al.*, 2011). Both lycopene and beta-carotene are antioxidant micronutrients that play a complementary role in regulating vital metabolic reactions, scavenging free radicals and reducing oxidative stress in the organism (Sanusi and Adebisi, 2009). They help in the process of food absorption. Carotenoids also give immunological effects such as reducing induced ultraviolet rays, strengthen defense cells, and play a role in the morphology of cell development. Carotenoids are generally divided into two main types: carotenoids and xanthophylls. Carotenoids are important in the body as they are a source of vitamin A. They also work as natural antioxidants that protect fats in the body from oxidation (Sikorski, 2007). Carotenoids are effective antioxidants that can remove free radicals from the environment or system by interacting with them or blocking their interactions. The double bonds rich in electrons are responsible for holding free oxygen and preventing oxidation (Britton, 1995), while carotenoids provide maximum protection from the effects of free radicals (Dutta *et al.*, 2005). The antioxidant activity of carotenoids, especially lycopene, is measured on the basis of its ability to suppress free radicals in samples of plant or animal cells that contain free radicals or what it provides to

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protect the cell from attacking radicals (Siva and Bhakta, 2010).

Acrylamide (ACR) is a white, crystalline, odorless substance that is formed in baked and fried foods as a result of exposure to high heat treatments. It has been found to have a negative effect on human health, as it is formed by heating at a temperature above 120°C as a result of Maillard reactions between the amino acid asparagine with glucose or any other sugar (Konings *et al.*, 2003). ACR is a chemical formed in cooked starchy foods such as potatoes and bread. High levels of acrylamide have carcinogenic, genotoxic, neurotoxic and hepatotoxic effects on living organisms.

MATERIALS AND METHODS

Lycopene extraction and purification: The tomatoes were dried according to the method of Poojary and Passamonti (2015) using an electric oven, after which lycopene was extracted from the tomatoes according to the method of Shi *et al.* (1999), using a mixture of hexane solvent, acetone, and ethanol in a ratio of 2:1: 1 in a row. The des-saponification process was carried out according to the method of Harwood *et al.* (1999), after which it was filtered after washing with acetone solvent, and the lycopene was dried under vacuum. The extracted lycopene was weighed and store it in special, light-proof containers at a low temperature.

Lycopene determination: The amount of lycopene extracted from tomatoes was estimated using a spectrophotometer, measuring the optical density at a wavelength of 5.04 nm, and using Caplanck hexane. The amount of lycopene was calculated using the Lycopene extinction coefficient (E%), which is 3150, according to the method of Chang and Liu (2007).

Preparation of laboratory animals: Adult male Albino rats were obtained from the College of Veterinary Medicine/University of Tikrit, at the age of 7-8 weeks. Their weights ranged between 125-160 grams, and they were randomly distributed into three groups with similar weights, with three animals in each group. The rats were fed for 5 days on the basic diet on a regular basis using a prepared diet according to (NRC, 1995). The necessary groups and solutions of acrylamide and lycopene were prepared as shown below:

- Control group: Without treatment (T1)
- The acrylamide treated group (T2): Prepare the solution at a concentration of 40 mg/kg/day.
- The group treated with acrylamide + lycopene T3: Prepare the solution at a concentration of 1.5 mg/kg/day.

Blood tests: After the end of the experiment, the animals were starved for 10 hours and anesthetized using chloroform. The rats were then dissected from the chest area and blood was drawn from the heart into two tubes, the first containing the anticoagulant EDTA to measure blood images, and the second devoid of it, containing 2-3 ml of blood, which was

centrifuged using a centrifuge. At a speed of 3000 rpm for 15 minutes, then the serum was taken and stored at a temperature of -20 °C until the necessary analyzes were performed, as in (Tietz, 2005).

Preparing histological sections: Tissue sections of the liver that were taken from experimental animals were prepared according to (Suvarna *et al.*, 2018).

Examination of histological slides: A compound microscope was used to examine tissue slides, and in order to explain some of the results of the study, a digital color image was taken using a Sony camera mounted on the microscope.

Statistical analysis: The data were statistically analyzed using the complete random design (CRD) system in the experimental system within the ready-made statistical program, and the averages were chosen according to Duncan's multiple range test to determine the significance of the differences between the averages of the factors affecting the studied characteristics at the level of (0.05).

RESULTS AND DISCUSSION

Lycopene extraction: We notice that after drying the tomatoes using an electric oven at a temperature not exceeding 80°C and extracting them with organic solvents, the amount of lycopene extracted amounted to 4.87 mg/100 g of the weight of the dried tomatoes according to the method of Shi and Maguer, (2000). The results agreed with what was reached by Shi and Maguer, (2000), reaching 5 mg/100 and 4.57 mg/100 g, respectively.

Lycopene purity: Figures (A1, B1) show the wavelengths of optical absorption of the lycopene sample extracted and separated using a silica gel column and the ideal sample. The highest absorbance of the lycopene sample extracted from dried tomatoes was at the wavelength of 5.06 nm. The result converged with the highest absorbance at the wavelength of the ideal sample of 5.04 nm. The minimum number of double bonds contained in these dyes is 7 bonds, which give the yellow color. The increase in the number of double bonds causes intensification of the color and an increase in the wavelength of absorption (Sikoriski, 2007). The red color of lycopene is due to it containing 13 double bonds, which led to the absorption of light at a wavelength of 5.04 nanometers for the extracted lycopene sample. The color of carotenoids is also affected by the type of solvent. It has been found that lycopene gives a yellow color in ether while it gives a dark red color in hydrogen sulfide (Fennema, 1996).

Effect on liver function: Table 1 shows the effect of lycopene on the liver enzyme values of male rats infected with acrylamide. We note from the results that there was a significant increase when fed acrylamide, as it was at (58.21, 77.57, and (181.64 international units/liter) compared to the control group (31,51,51, 77 and 152.45 international units/liter, respectively. We also notice a significant decrease



in the values of the AST, ALT, and ALK enzymes for the group of rats fed lycopene (T3), as they were (45.98, 65.51, and 166.61 international units/liter compared to the group infected with acrylamide (T2).

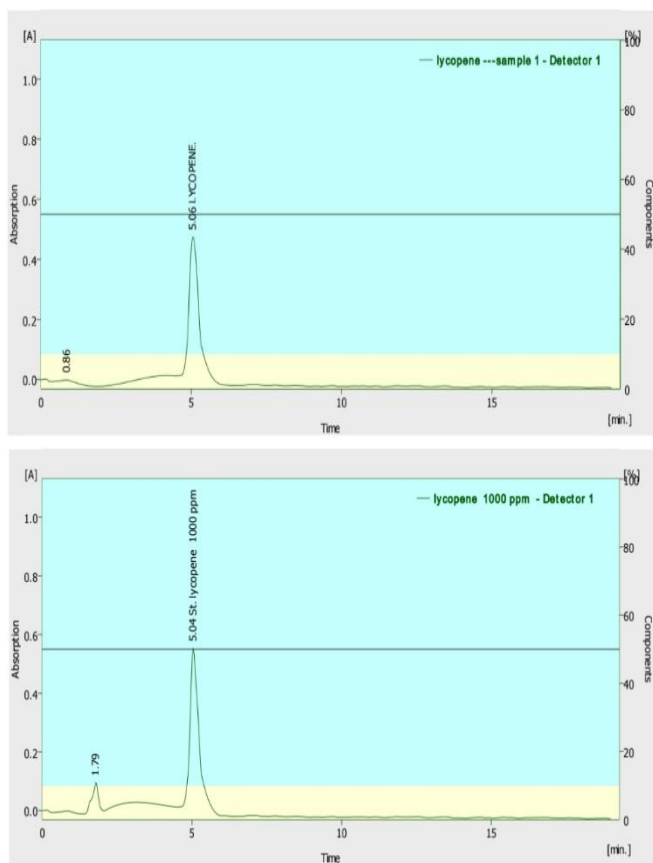


Figure 1. Optical absorbance of extracted lycopene sample A and ideal sample B.

Acrylamide causes cell damage, which increases the permeability of membranes and a defect in the vital transport of cells. This defect causes an increase in the transfer of liver enzymes into the blood (Awad *et al.*, 1998). Moss and Rosalki (1996) also indicated that adding acrylamide causes a defect or disturbance in Liver functions.

Due to liver membrane damage, it leads to the release of liver enzymes into the blood circulation, and their high level in the serum is evidence of liver damage. It is known that increased activity of the enzymes AST and ALT in blood plasma results from damage to the liver and the leaching of these enzymes from inside the cell to the outside due to oxidative stress by acrylamide, and since lycopene is an effective antioxidant that works to protect the liver from the destructive damage of free radicals, thus reducing Liver damage, which leads to the leaching of enzymes out of the cells.

The results agreed with Fatma and Rabab (2019), who indicated that taking lycopene at a concentration (10

mg/kg/day) and acrylamide at a concentration (25 mg/kg/day) orally for 6 weeks led to a decrease in the level of glutathione (GSH) when compared with... Control group. Conversely, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and malondialdehyde (MDA) levels were increased as a result of acrylamide-induced effects. After treatment with lycopene, it was observed that there was an increase in the level of the hormone GSH and a decrease in the levels of AST, ALT and MDA. It was found that lycopene reduces acrylamide - liver damage due to the powerful antioxidants Properties of lycopene.

Meydan *et al.* (2011) pointed out the protective effect of lycopene and protecting the liver from damage when using radiotherapy, which led to an increase in free radical levels and a decrease in the activity of glutathione (GSH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) levels, on the contrary. Oral administration of lycopene significantly increased the rate of antioxidant enzymes. An increase in liver enzymes occurs with oral administration of acrylamide, It could be due to the effect of this substance on the metabolic processes of the liver cells and the inhibition of the enzymatic activity responsible for the metabolic processes of the liver, which leads to damage to the liver cells and the release of enzymes outside the cells into the bloodstream, thus increasing their values, especially ALT, which indicates a defect in the functioning of the liver (Korkmaz *et al.*, 2010).

Table 1. The effect of oral administration of lycopene on the activity of liver enzymes in rats exposed to acrylamide for 28 days.

Groups	Average of liver enzymes IU/L		
	AST	ALT	ALK
T1	0.26±31.51 b	0.94±51.77 b	1.27±152.45 b
T2	0.59±58.21 a	0.94±77.57 a	1.92±181.64 a
T3	0.64±45.98 c	0.14±65.51 c	0.79±166.61 c

Control group

Liver tissue: The results of the study for the control group showed that the liver tissue appeared normal, with the hepatic cells symmetrical around the central vein in the form of radial cords (Figure 1). Histological sections also showed that the hepatic cells contain a central nucleus and normal pink cytoplasm (Figure 1). The hepatic sinusoids between the liver cells appeared normal, with no expansion between the cells (Figure 1).

The portal region also appeared normal, with the hepatic vein containing some blood cells, with the hepatic artery having normal symmetry, as well as the bile duct, which contained normal cuboidal epithelial tissue (Figure 3). As for Coover cells, they appeared normally distributed in the hepatic sinusoids.



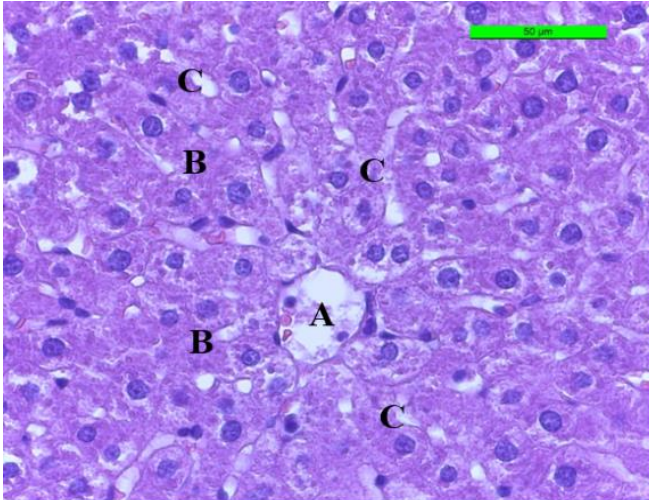


Figure 2. Liver tissue; Central vein (A), radially symmetrical hepatocytes with central nucleus (B), hepatic sinusoids (C) H&E 50X.

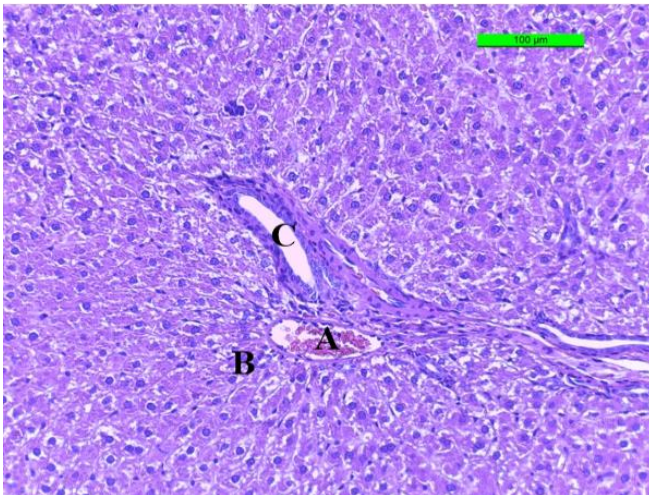


Figure 3. Liver tissue; Slightly engorged hepatic vein (A), hepatic artery (B), hepatic venule, bile duct (C). H&E 20X.

Liver tissue of the infected group: The liver tissue of the infected group, it showed an asymmetry of the hepatic cells around the central hepatic vein, with its expansion being very large and containing red blood cells and some protein substances (Figure 4, 5) and an increase in the thickness of its wall (Figure 6). The sections also showed the presence of blood bleeding. Severe with infiltration of inflammatory cells in the portal area. As for the hepatic sinusoids, they appeared very narrow or disappeared due to an increase in the size of the hepatic cells (Figure 7), with degeneration of some of them (Figure 8). It was also noted that the percentage of Coover cells present in the hepatic sinusoids decreased.

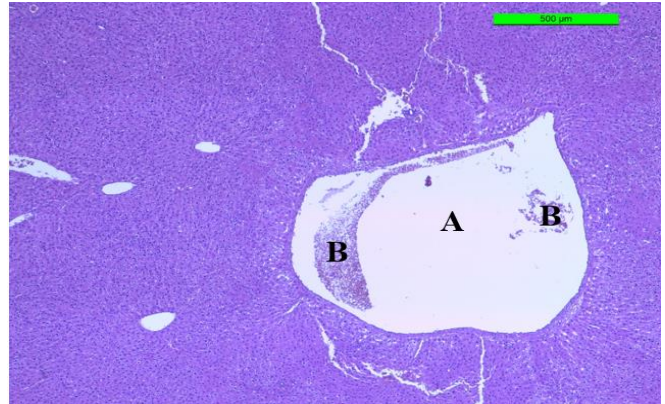


Figure 4. Liver tissue; The central vein is very widely dilated (A), protein materials and red blood cells (B). H&E 5X.

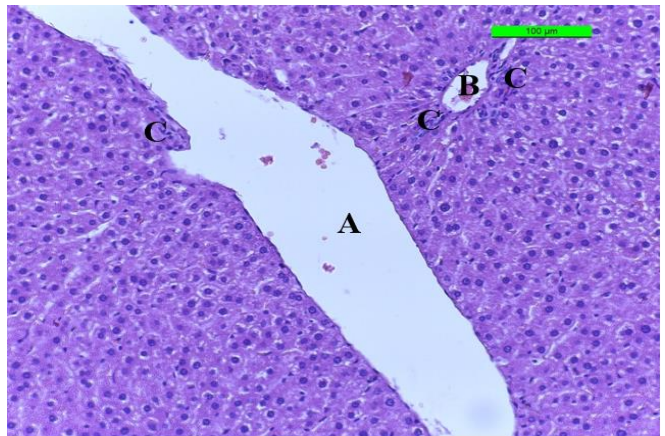


Figure 5. Liver tissue; Very extensive central venous dilatation (A), central venous congestion (B), hepatocyte infiltration (C). H&E 20X.

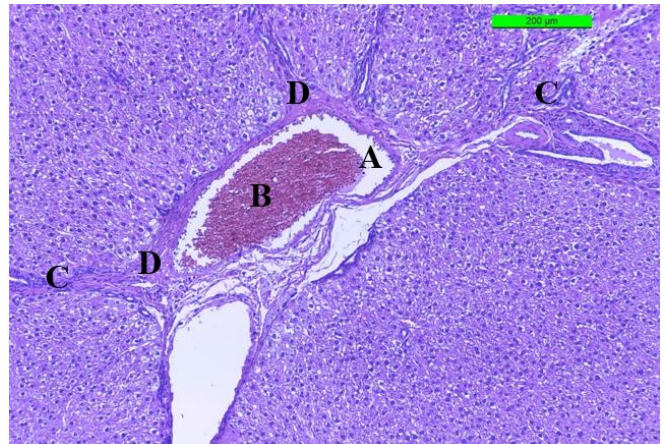


Figure 6. Liver tissue; Very extensive central vein dilatation (A), central vein congestion (B), hepatocyte infiltration (C), central vein wall thickening (D). H&E 10X.



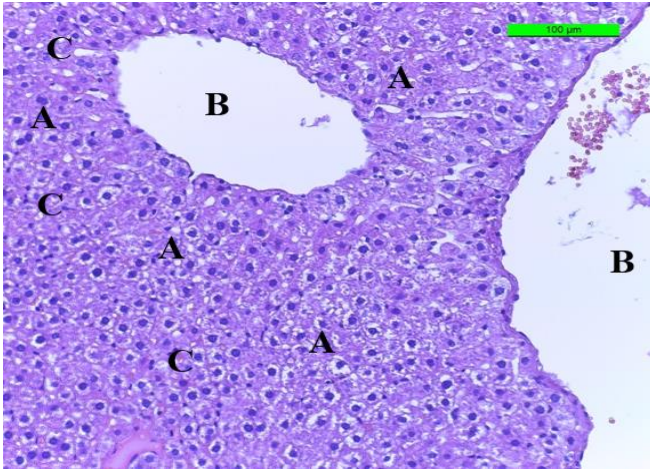


Figure 7. Liver tissue; Radial asymmetry of the hepatocytes (A), dilatation of the central vein (B), and stenosis of the hepatic sinusoids (C). H&E 20X.

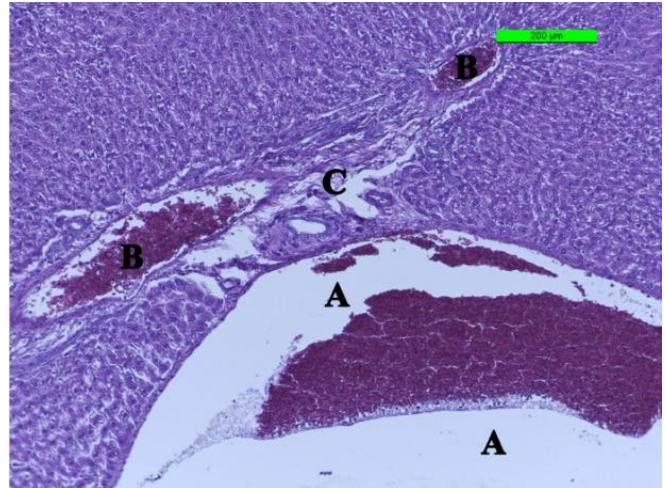


Figure 9. Liver tissue; the central vein is surrounded by dark-stained nuclei (A) and the portal vein of the liver contains decomposed blood (B). The hepatocytes appeared normal and contained pale-stained nuclei (C). H&E 20X.

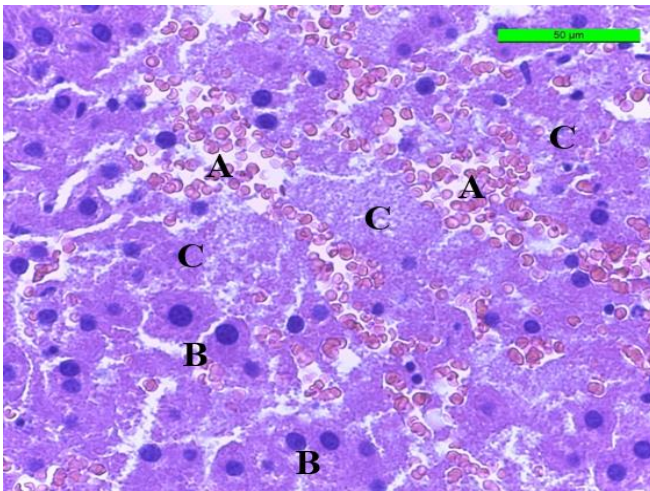


Figure 8. Liver tissue; Hemorrhage between hepatocytes (A), increase in the size of the nucleus and hepatocytes (B), degeneration and destruction of liver cells (C). H&E 50X.

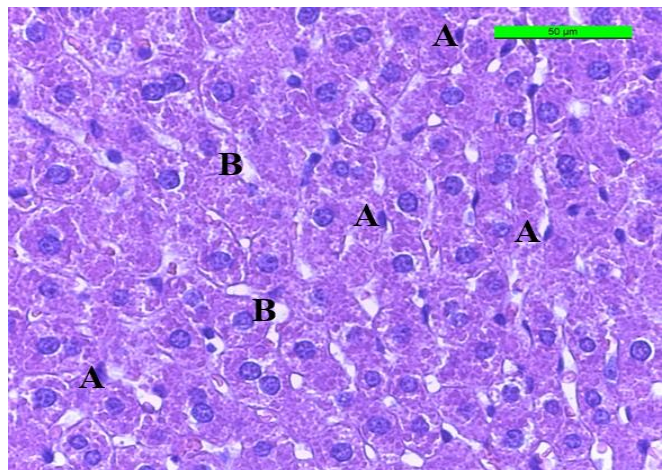


Figure 10. Liver tissue; Coffor cells in the hepatic sinusoids are regularly distributed (A), the hepatic sinusoids are slightly dilated (B). H&E 50X.

The effect of a concentration of 1.5 mg/kg of lycopene on the liver tissue of male rats: The histological reading upon oral administration of lycopene in group T5 showed that the central vein in the hepatic lobule is surrounded by rows of polygonal hepatocytes containing dark spherical nuclei, and the blood sinusoids contain Coover cells, and these sinusoids are continuous with the central vein at its edges (Figure 9), the central vein in The 0central lobule contains decomposed blood (Figure 9), and the blood sinusoids are slightly expanded and contain regularly arranged Cover cells (Figure 10). As for the rows of hepatic cells, they appear normal and contain nuclei of pale pigment.

The treatment with acrylamide showed significant hepatotoxicity, and it was characterized by expansion and congestion of the hepatic sinusoids and the central vein, necrotic inflammatory foci, and the hepatic lobule. The acrylamide group also showed inflammatory cell infiltration, hepatic cell necrosis, and areas of bleeding. [Fatma and Rabab \(2019\)](#) also confirmed that histological anatomy of liver examinations showed widening and congestion of the central vein and hepatic sinusoids, Infiltration of inflammatory cells, and necrosis of hepatic cells in the group exposed to acrylamide, in addition to hypertrophic necrosis of liver



tissue. After treatment, lycopene showed its ability to improve and restore liver enzymes and oxidation markers (GSH) and malondialdehyde to normal levels. Tarfa and Mona (2021) indicated in their study on experimental animals that there was an improvement in fatty liver tissue as a result of eating foods rich in fat when they were given lycopene at concentrations of 10, 25, and 50 mg/g. Lycopene is a natural pigment, synthesized by plants and microorganisms. Red fruits and vegetables are the most common sources of lycopene, which show the highest antioxidant activity among all dietary carotenoids. Therefore, at present, interest has begun in the role of lycopene on human health through its ability to protect against oxidative damage (Rao *et al.*, 1999). Jiang *et al.* (2016) found that treatment with lycopene was able to inhibit the elevation of liver enzymes and signs of liver damage, suggesting that lycopene's antioxidant activity plays a role in the liver protection mechanism and the reduction of acrylamide and its toxic effects on the liver.

Conclusion: In conclusion, lycopene as an antioxidant phyto-component, can protect liver against damages caused by acrylamide (or acrylic amide). Tomatoes can be considering as functional vegetable for protecting liver from damages of chemicals such as acrylamide. In future studies, investigation on acrylamide as a bio-antioxidant can be considered as a replacement of chemical antioxidant.

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Ethical statement: all procedure of study, especially in vivo part of study was in according to international ethical rules and also under monitoring of ethical committee of University of Tikrit.

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Informed consent: N/A

Consent for publication: All authors submitted consent to publish this research article in JGIAS.

SDGs addressed: Good Health and Well-being, Responsible Consumption and Production, Life on Land.

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