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Original Article

The Potency of Tomato Paste (*Lycopersicon esculentum*) on the Expression of Interleukin-2 in Hepar of Mice Exposed to Borax

Puput Ade Wahyuningtyas[1], Djoko Legowo[2], Eka Pramyrtha Hestianah[2], Hani Plumeriastuti[2], Dinar Adriaty[1,*], Iswahyudi[1], Ratna Wahyuni[3], Medhi Denisa Alinda[4], Cita Rosita Prakoeswa[4]

[1]*Institute of Tropical Disease, Airlangga University, Mulyorejo Street, Surabaya 60115, Indonesia*
[2]*Faculty of Veterinary Medicine, Airlangga University, Mulyorejo Street, Surabaya 60115, Indonesia*
[3]*Department of Health, Faculty of Vocational Studies, Airlangga Univesity, Surabaya 60115, Indonesia*
[4]*Department of Dermatology and Venereology, Faculty of Medicine Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya 60286, Indonesia*

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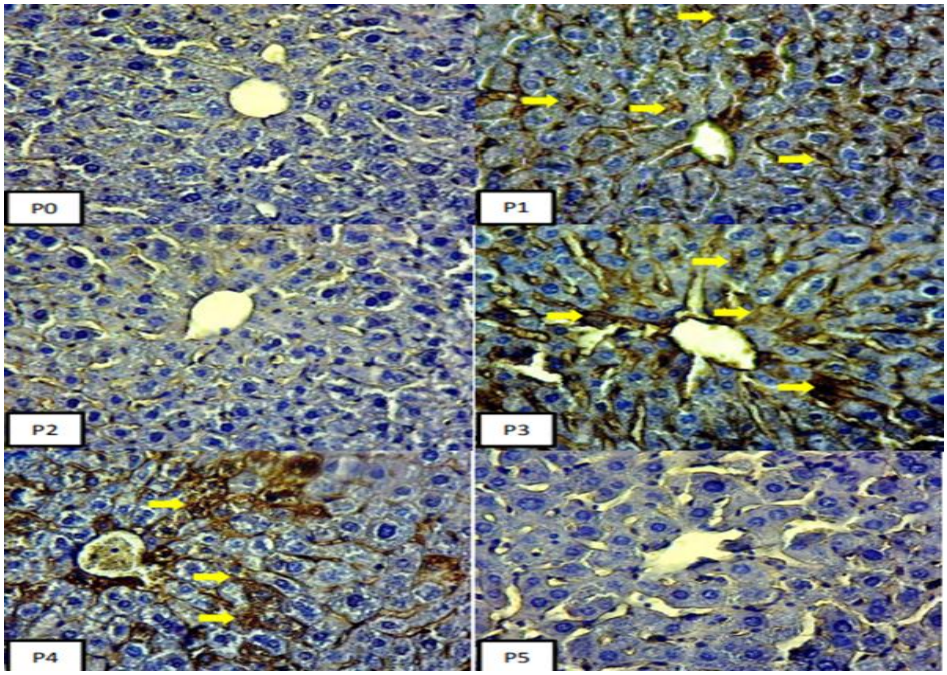
ABSTRACT

The food industry in Indonesia still uses chemicals in food products. Borax is a chemical substance that has long been abused by the food industry to reach high profits at low production costs. This toxic material will cause damage and necrosis of liver cells. This research aimed to investigate the protective effect of tomato paste on liver histopathological changes exposed to sodium tetraborat or borax. This study used thirty-six healthy male mice two months old with a body weight of 20 grams that were divided into six treatment groups for 17 days and initial three days treatment was as follows. Group P0 has been given sterile aqua dest for 0.1 ml/20 g, group P1 has been given borax for 5.6 mg/20 g, group P2 has been given tomato paste for 230 mg/20 g, and groups P3, P4, and P5 were prevented by tomato paste for 110 mg/20 g, 230 mg/20 g, and 350 mg/20 g daily. For the next 14 days, groups P3, P4, and P5 have been exposed to borax every day as much as 5.6 mg/ 20 g while groups P0, P1, and P2 had the same treatment as before. On the 18th day, the mice were sacrificed by cervical dislocation, followed by liver sample collection. This research showed that lycopene in tomato paste likely protected the liver from borax because the IL-2 expression was decreased. It can be concluded that P5 group offers the best protection against borax-exposed liver. It is necessary to carry out further research regarding the protection of tomato paste on borax exposure in other organs.

* Corresponding author: Dinar Adriaty
✉ E-mail: dinadria@gmail.com

GRAPHICAL ABSTRACT



Introduction

The Indonesian food industry still heavily uses chemicals in food. The chemical is often abused in the food industry to generate high profits at low production costs is sodium tetraborate or borax. Borax is a chemical substance that is widely abused as a food preservative, making food chewier and improving the appearance of food products. Based on the survey analysis in 2020 with 34 samples taken in 17 sub-districts, all meatball skewers sold in Yogyakarta, Indonesia contain borax [1]. Lots of food industries still use borax even though the goverment in Indonesia has published health regulation regarding food additives where borax is included as an ingredient prohibited to be added to food because it is dangerous and toxic. Borax has a high level of solubility, so its distribution quickly spreads throughout the body's tissues. The organs most often affected by side effects from giving borax are the liver and kidney. The liver is an organ that functions as a detoxification of oxic material. Almost all drugs or substances that enter orally experience metabolism in the liver. Toxic substances that have been consumed will enter the liver and undergo a detoxification process. These toxic materials will cause damage and necrosis. Borax entering the body in the ROS form will cause several responses, i.e. borax will seek ion pairs that might cause damage to the cell membrane and activate the Kupffer cell and TLR4 as pro-inflammatory to express IL-2 [2]. Based on the research conducted in 2013, it was found that giving juice tomato after Carbon Tetrachloride (CCl_4) induction showed regeneration in almost all hepatocytes in the liver [3]. Tomatoes contain antioxidants in the form of lycopene ability to repair damage to body cells caused by free radicals. In tomatoes processed into tomato paste contains four times more than in fresh tomatoes, juice, sauce and others. This is due to Lycopene is insoluble in water and is tightly bound in fiber. Lycopene ability to effective protective method against cancer, diabetes mellitus, cardiac complications, oxidative stress-induced dysfunction, inflammatory events, skin and bone diseases, liver, and neurological and reproductive disorder [4].

The study to determine the level of expression of IL-2 in the administration of tomato paste on the histopathological appearance of the mouse liver exposed to borax after administration of tomato paste, with the hypothesis that IL-2 is not expressed in the liver of mice exposed to borax previously protected with tomato paste. Free radical exposure from borax is found in the ROS (reactive oxygen species) form [5].

Borax is transformed into boric acid in the body, which is impossible to metabolize. Borax, or boric acid, entering for approximately 81-95% is completely absorbed between 24 and 96 hours. Long exposure will lead to liver and kidney dysfunction [6]. Borax, a toxic substance, will damage cell membranes if it enters the body. Therefore, the Damage Associated Molecular Pattern (DAMP) molecules will activate TLR4 as pro-inflammatory on the cell surface. Each TLR has the same structure and will transmit signals via NF-Kb. Different TLRs trigger specific biological responses due to the differential involvement of adaptor molecules containing Toll/interleukin 1 domain, including MyD88 and the TIR-domain-adapter interferon-β (TRIF). NF-Kb is immediately translocated to the nucleus to induce appropriate gene expressions. NF-Kb excretes P65 and P50 that initiate cytokine excretion in the Kupffer cell. During the inflammation process, IL-2, TNFα, IFN$_\gamma$, and IL-12 cytokines will be excreted. IL-2 has an active role in suppressing Th17 differentiation (pro-inflammatory) and improving Kupffer cell ability in phagocytizing toxic substances such as borax or others [7]. Tomato paste has a working mechanism in preventing inflammation since it has lycopene, which prevents Reactive Oxidative Stress (ROS) by producing ions and inhibiting ROS formation directly by ROS scavenging materials or indirectly by its ability to inhibit RAD enzyme expression, which produces NADPH oxidase. Furthermore, lycopene can inhibit TLR4, known as pro-inflammatory. Decreased TLR4 transport to lipid rafts or disrupted TLR4 adapter association leads to TLR4 inactivation [8]. Therefore, it is necessary to prove in this study regarding the protective effect of tomato paste against histopathological changes in liver exposed to sodium tetraborate or borax.

Materials and Methods

Materials

This research is an experimental laboratory study. The samples used in this study were 36 mice (Mus musculus) divided into six mice in each treatment group with the criteria of male mice, healthy, weight 20-30 grams and 2 months old have complete and well-developed organs. The materials used in this research are borax from chemical store, packaged tomato paste (Leggo's, 021642139), aquadest steril (Otsuka, DKL991870534341), feed given to mice in the form of chicken feed 511 (Charoen Pokphand, 3121-18/100/1), Canada Balsam (Merck, 8007-47-4), and anti-IL-2 monoclonal antibody (NovusBio) for the immunohistochemical examination. While the tools used in this study are cages for experimental animals, sonde needles for give borax and tomato paste, necropsy equipment, organ storage, paraffin block making tools, microtome, water bath, and Olympus® CX-21 microscope.

Preparation of experimental animals

Mice are divided by numbering then taken randomly according to the number drawn. Mice were put into five cages each cage containing six mice. Mice were acclimatized for seven days and given food and drink ad libitum, for three days before treatment, mice with treatment codes P3, P4, and P5 were given tomato paste orally according to the prescribed dose.

Treatment

Borax dosage 40 mg of borax for one rat will cause the liver to microscopically undergo hydropic degeneration and fibroblast proliferation and macroscopically be enlarged with brown colour [9]. Toxic effects of borax can be achieved using a subchronic dosage for 14 treatment days. The borax solution was administered orally for 0.1 ml/ 20 g rat weight daily using a probe. Tomate paste dosage that lycopene administration for 4 mg/kg adult Sprague-Dawley rat positively affected the diameter of testicular seminiferous tubules exposed to filtered cigarettes [10]. The mice were

categorized into six treatment groups, i.e. P0 has been given sterile aqua dest for 0.1ml/20g for 14 days, P1 has been given borax for 5.6 mg/20 g for 14 days, P2 has been given tomato paste (Leggo's) for 230 mg/20 g for 14 days, and groups P3, P4, and P5 are the groups where mice were protected by tomato paste for three days, and given tomato paste for 110 mg/20 g, 230 mg/20 g, and 350 mg/20 g, for the next 14 days before exposed to borax for 5.6 mg/20 g. On the 18th day, mice were necropsied to sample the liver, and the samples were made for histopathological preparations by immunohistochemistry method with anti-IL-2 monoclonal antibody.

Analysis

In liver histopathological preparations with immunohistochemical staining with IL-2 monoclonal antibodies, tissue expressing IL-2 will be brown. Observations were made with a 400x magnification microscope, and then IL-2 expression was measured in liver tissue with an Immunoreactivity Score method. Observation of the histopathological picture of the liver of mice exposed to borax showed no expression of IL-2 due to protection from tomato paste. The results in the form of data are presented in the form of mean rank and SD (Standard Deviation) through SPSS (Statistical Product and Service Solutions) software. Data were analyzed statistically using the Kruskal-Wallis test. If there is a significant difference between the study groups ($p < 0.05$), they are proceed with the Man-Whitney test.

Results and Discussion

The mean score of Interleukin 2 (IL-2) expression and standard deviation calculation results is presented in Table 1.

Statistical analysis using the Kruskal-Wallis test showed a significant difference ($p>0.05$). Subsequently, the Mann-Whitney test obtained the mean score of histopathological illustration of the liver expressing the highest IL-2 on the P1 (3.6±0.21) group with 5.6 mg/20 g weight/day dosage compared with negative control group P0 (0.46±0.16). The P2 and P5 groups were significantly different ($p>0.05$) from P1, P3 and P4 groups. This indicates that the expression of IL-2 in group P2 and P5 which has a mean value of (0.7±0.2) and (0.63±0.15), similar to the negative control group P0 (0.43±0.12).

Based on the histopathological picture of the liver stained with immunohistochemistry (Figure 1), the expression of IL-2 cytokines showed a decrease in IL-2 expression levels in the group given preventive lycopene in tomato paste. Tomato paste has a working mechanism for preventing inflammation since it contains lycopene, which prevents Reactive Oxidative Stress (ROS) by inhibiting TLR4, known as pro-inflammatory [11].

In the liver histopathological illustration examination, IL-2 expression also shows body immune responses from Kupffer cell activation when toxic substances enter the body. The IL-2 expression intensity index was found on immunohistochemistry as indicated by brown colour in sinusoid hepar under a microscope with 400x magnification. IL-2 expression will be seen when the organs are exposed to borax. IL-2 expression was very distinctive in the P0 and P1 treatment groups, where IL-2 expression in the P1 group was higher. Borax is a toxic substance that activates TLR4 as a pro-inflammatory on the cell surface when it enters the body. Each TLR has the same structure and will transmit signals via NF-Kb. Different TLRs trigger specific biological responses through differential engagement of Toll/interleukin-1 domain-containing adapter molecules, including MyD88 and the TIR domain adapter interferon-β (TRIF). NF-Kb is immediately translocated to the nucleus to induce appropriate gene expressions. NF-Kb excretes P65 and P50 that initiate cytokine excretion in the Kupffer cell. Cytokine is a mediator of peptide form produced from various cells. IL-2, TNFα, IFN$_\gamma$, and IL-12 are cytokines excreted during the inflammation process. IL-2 has an active role in suppressing Th17 differentiation (pro-inflammatory) and improving Kupffer cell ability in phagocytizing toxic substances such as borax or others [7].

During the IL-2 cytokine expression examination, IL-2 expression was reduced in groups with a preventive action of lycopene in tomato paste.

Table 1: Mean score ± SD of IL-2 expression of rat livers on all treatments

Treatment		IL-2 expression (mean + SD)
P0	Sterile aqua dest 0.1ml	
P1	Borax 5.6 mg	
P2	Tomato Paste 0.3 g	
P3	Tomato Paste 0.11 g + Borax 5.6 mg	
P4	Tomato Paste 0.23 g + Borax 5.6 mg	
P5	Tomato Paste 0.35 g + Borax 5.6 mg	

**Different superscript symbols (^{a,b,c}) in each column indicate that there are significant differences in the data in each treatment based on the test.*

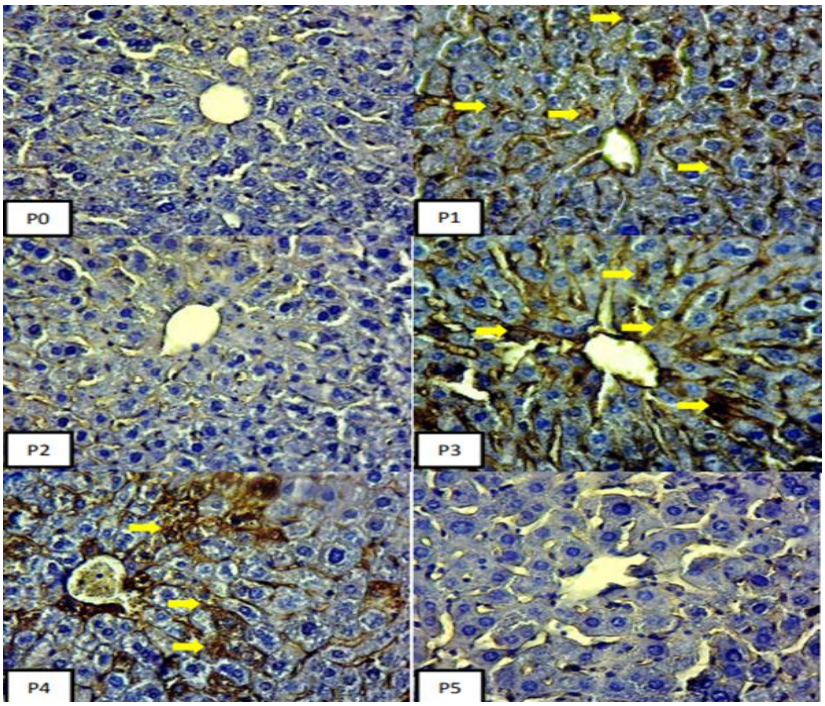


Figure 1: Histopathological description of IL-2 expression by immunohistochemical staining in the central venous area

Evidently, the P5 had no significant different mean score than the negative control group, i.e. P0. It shows that inhibition of TLR4 as pro-inflammatory will increase when exposed to toxic substances. This study result aligns with the viewpoint of research that lycopene can inhibit TLR4 as pro-inflammatory on signals mediated by TLR4, which eventually will activate Nuclear Factor Kappa B (NF-KB) [12]. Signalling activation of NF-κB pro-inflammatory is involved in cytokine production from tumor-promoting/inflammation, and thus triggering cell proliferation. Fundamentally, each TLR has the same structure and will transmit signals via NF-Kb. Different TLRs trigger specific biological responses through differential engagement of Toll/interleukin-1 domain-containing adapter molecules, including MyD88 and the TIR domain adapter interferon-β (TRIF). The degradation of NFKb inhibitor I-Kb releases NF-Kb that immediately translocates to the nucleus to induce appropriate gene expressions. NF-Kb excretes P65 and P50 that initiate the excretion of IL-2, TNFα, IFN_{γ}, and IL-12 cytokines. Another viewpoint on lycopene can prevent Reactive Oxidative Stress (ROS) by producing ions and inhibiting ROS formation directly by ROS scavenging materials or indirectly by its ability to inhibit RAD enzyme expression, which produces NADPH oxidase. Furthermore, lycopene can also inhibit TLR4, known as pro-inflammatory. Lycopene evidently reduces TLR4 complex formation aided by an adapter on the membrane. It is caused by TLR4 transportation decrease to

lipid rafts or disrupted TLR4 adapter association, leading to TLR4 inactivation [8].

Conclusion

The study results conclude that lycopene administration in tomato paste in the P5 group with a dosage of 350 mg/20 g weight/day as prevention for mice exposed to borax could reduce liver damage, observed from decreased Kupffer cell activation and IL-2 expression, balanced with the indicator of hepatocyte necrosis level decrease. Compared to the negative control group (P0), tomato paste administration, particularly in group P5, had a protective power on the liver exposed to borax. It is necessary to carry out further research regarding the protection of tomato paste on borax exposure in other organs. Further research is needed regarding the potential of tomatoes in other forms such as tomato extracts, juices, sauces, jams, etc. to determine the potential of tomatoes in preventing organ damage due to exposure to borax.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

ORCID

Puput Ade Wahyuningtyas
https://orcid.org/0009-0002-5378-8551
Djoko Legowo
https://orcid.org/0000-0003-0099-8782
Eka Pramyrtha Hestianah
https://orcid.org/0000-0001-8679-040X
Hani Plumeriastuti
https://orcid.org/0000-0002-4540-811X
Dinar Adriaty
https://orcid.org/0000-0002-2766-2982
Iswahyudi Iswahyudi
https://orcid.org/0009-0008-7057-302X

References

[1]. Harimurti S., Bariroh I.H., Setiyawan A., Permatasari R.I., Putri F.D., Fajriana L.Y., Identification of the spread of borax use in meatball skewers in Bantul District, Special Region of Yogyakarta, *Jurnal Farmasi Sains dan Komunitas*, 2020, **17**:120 [Crossref], [Google Scholar], [Publisher]

[2]. Dixon L.J., Barnes M., Tang H., Pritchard M.T., Nagy L.E., Kupffer cells in the liver. *Comprehensive Physiology*, 2013, **3**:785 [Crossref], [Google Scholar], [Publisher]

[3]. Sari Tappi E., Lintong P., Loho L. Gambaran Histopatologi Hati Tikus Wistar Yang Diberikan Jus Tomat (Solanum Lycopersicum) Pasca Kerusakan Hati Wistar Yang Diinduksi Karbon Tetraklorida (Ccl_4). *eBiomedik,* 2013, 1 [Crossref], [Google Scholar], [Publisher]

[4]. Imran M., Ghorat F., Ul-Haq I., Ur-Rehman H., Aslam F., Heydari M., Shariati M.A., Okuskhanova E., Yessimbekov Z., Thiruvengadam M., Hashempur M.H., Lycopene as a natural antioxidant used to prevent human health disorders, *Antioxidants*, 2020, **9**:706 [Crossref], [Google Scholar], [Publisher]

[5]. Chen D., Song M., Mohamad O., Yu S.P., Inhibition of Na+/K+-ATPase induces hybrid cell death and enhanced sensitivity to chemotherapy in human glioblastoma cells, *BMC cancer*, 2020, **14**:716 [Crossref], [Google Scholar], [Publisher]

[6]. Mitrut I., Scorei I.R., Manolea H.O., Bita A., Mogoanta L., Neamtu J., Bejenaru L.E., Ciocilteu M.V., Bejenaru C., Rau G., Mogosabu G.N., Boron-containing compounds in Dentistry: A narrative review, *Romanian Journal of Morphology and Embryology*, 2022, **63**:477 [Crossref], [Google Scholar], [Publisher]

[7]. Capobianco M.P., Cassiano G.C., Furini A.A.C., Melo L.M.S., Domingos C.R.B., Machado R.L.D.

Human Interleukin 2 (IL-2) Promotion of Immune Regulation and ClinicalOutcomes: A Review. *Journal of Cytokine Biology*, 2016, **1**:109 [Crossref], [Publisher]

[8]. Zou J., Feng D., Ling W.H., Duan R.D., Lycopene suppresses proinflammatory response in lipopolysaccharide-stimulated macrophages by inhibiting ROS-induced trafficking of TLR4 to lipid raft-like domains, *The Journal of nutritional biochemistry*, 2013, **24**:1117 [Crossref], [Google Scholar], [Publisher]

[9]. Tatukude P., Loho L., Lintong P. Gambaran histopatologi hati mencit swiss yang diberi air rebusan sarang semut (Mymercodia pendans) paska induksi dengan carbon tetrachlorida (CCL_4). *eBiomedik*, 2014, **2** [Crossref], [Google Scholar], [Publisher]

[10]. Sugeng S.U., Tiono H., Anandaputri V.N., Pengaruh Pasta Tomat (Solanum Lycopersicum) Terhadap Diameter Tubulus Seminiferus Mencit (Mus Musculus) Galur DDY Yang Terpajan Asap Rokok Berfilter, *Maranatha Journal of Medicine and Health,* 2010, **10** [Google Scholar], [Publisher]

[11]. Pi Y., Zhang L.L., Li B.H., Guo L., Cao X.J., Gao C.Y., Li J.C., Inhibition of reactive oxygen species generation attenuates TLR4-mediated proinflammatory and proliferative phenotype of vascular smooth muscle cells, *Laboratory investigation*, 2013, **93**:880 [Crossref], [Google Scholar], [Publisher]

[12]. Ip B.C., Wang X.D., Non-alcoholic steatohepatitis and hepatocellular carcinoma: implications for lycopene intervention, *Nutrients*, 2014, **6**:124 [Crossref], [Google Scholar], [Publisher]

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