



RESEARCH ARTICLE

Effect of *Eucheuma cottonii* extract on interleukin-1 level and caspase-3 expression in borax-induced rat's hepatocyte

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ABSTRACT

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Borax is a hepatotoxic substance and induces oxidative stress in hepatocytes. It may trigger the expression of pro-inflammatory cytokines like Interleukin-1 and apoptotic signals like Caspase-3. Extract of the seaweed *Eucheuma cottonii* contains carotenoids and flavonoids which suppress oxidative stress in hepatocyte. This study was conducted to determine the effect of *E. cottonii* extract on borax-induced Interleukin-1 and Caspase-3 expression in rat hepatocyte cells. This research employs a posttest-control group design. Twenty Wistar male rats were separated into four groups: control group (K1) received no intervention, K2 received Na-CMC 0.5% for 17 days and Borax 40 mg/200 g on the fourth day every hour for 14 days, P1 and P2 groups were administered *E. cottonii* extracts at doses of 600 mg/200 g and 1200 mg/200 g for 17 days, respectively, followed by borax induction at a dose of 40 mg/200 g on the fourth day with a one-hour interval for 14 days. Interleukin-1 (IL-1) levels were measured with ELISA, while caspase-3 expression was measured with PCR. For the statistical analysis, Kruskal Wallis and Mann Whitney were used. The level of IL-1 in P2 was significantly lower than in K2 ($p < 0.05$). Caspase-3 expression was significantly lower in P1 and P2 than in K2 ($p < 0.05$). IL-1 level and caspase-3 expression of hepatocytes are affected by administration of *E. cottonii* extract. The extract has the ability to prevent inflammation and hepatocyte apoptosis.

1. Introduction

Borax is a chemical substance that is widely used as food preservatives, enhancing color and texture of food. According to Regulation of the Minister of Health No. 033 of 2012 that the use of borax as a food additive is prohibited because borax is toxic to cells (Kemenkes-RI, 2012). Although its use has been prohibited, borax is still present in some foods. Using an acid-base titration test, the 2020 research conducted in the heart of Semarang revealed that meatball samples contained 3.02% borax (Eva, 2020). Borax abuse causes dysfunction in the brain, liver, and kidneys (Adinugroho,

2013). The liver, which is responsible for detoxifying toxins, is extremely vulnerable to damage (Guyton and Hall, 2016). Rat hepatocytes underwent necrosis when borax was administered for 14 days at a dose of 40 mg/kg BW to rats of the Wistar strain (Neca, 2021).

Borax produces free radical ions $4B(OH)_4^-$ in the body. This ion combines with the ion of an unsaturated fatty acid to produce hydroxyl radicals ($OH\cdot$). Hydroxyl free radicals induce oxidative stress in hepatocytes, which manifests as lipid peroxidation, protein modification, and DNA damage. The mitochondrial pathway is activated by apoptotic signal triggered by DNA stress. Hepatocyte apoptosis is characterized

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by an increase in caspase-3 expression, which is an executor caspase in the process of cell apoptosis (Kumar and Abbas, 2015). In addition, free radicals increase the secretion of proinflammatory cytokines such as IL-1, which contributes to the inflammatory process in damaged cells by stimulating the migration of neutrophils and monocytes. Inflammation increases the production of free radicals, which can exacerbate hepatocyte damage (Abul *et al.*, 2016). To prevent the harmful effects of free radical exposure on the body, it is essential to provide a balanced antioxidant. As a maritime nation, Indonesia has tremendous potential for developing natural antioxidants derived from seaweed. Compared to conventional antioxidants, seaweed has several advantages, including easy cultivation, relatively lower prices, simple processing, and absence of adverse side effects (KKP-RI, 2018). Most participants in the Pakistani developing country study believed that herbal medicine was safe and inexpensive (Kanwal and Sherazi, 2017).

Eucheuma cottonii is the most widely cultivated type of seaweed. There are protein, fiber, carbohydrates, essential fatty acids, minerals, and chlorophyll among the bioactive compounds present (Yuniarti *et al.*, 2020). In a phytochemical analysis of *E. cottonii*, alkaloids, flavonoids, and saponins were identified (Hudaifah *et al.*, 2020). The inhibitory concentration 50 (IC₅₀) value for *E. cottonii* seaweed extract is 23.15 g/mL. This value falls under the category of extremely powerful antioxidant activity. Bioactive compounds in *E. cottonii* play a role in the production of antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) (Yanuarti *et al.*, 2017). Other compounds such as carotenoids are also present in *E. cottonii* (Palawe and Tumonda, 2018). The role of seaweed as an antioxidant on IL-1 cytokine secretion and caspase-3 expression has not been fully elucidated. Therefore, this study compared the effect of seaweed extract *E. cottonii* at doses of 600 mg/200 g and 1200 mg/200 g on IL-1 level and caspase-3 expression in hepatocytes of borax-induced rats.

2. Materials and Methods

2.1. Experimental design

This study employed an experimental design with a posttest-only control group. This study was conducted at the Stem Cell and Cancer Research Laboratory (SCCR) of Universitas Islam Sultan Agung, Semarang. Twenty male Wistar rats (*Rattus norvegicus*) weighing between 200 and 250 g and aged 8 weeks were divided into four groups. The control group (K1) received no interventions, while the experimental group (K2) received Na-CMC 0.5% orally once a day for 17 days and borax 40 mg/200 g on the fourth day with a

one-hour interval for 14 days. Treatment groups (P1 and P2) received *E. cottonii* extracts at doses of 600 mg/200 g and 1200 mg/200 g in 0.5% Na-CMC orally once a day for 17 days, as well as Borax at a dose of 40 mg/200 g on the fourth day with a one-hour interval for 14 days. The bioethics committee of the Faculty of Medicine at Universitas Islam Sultan Agung, Semarang has approved this study (No. 173/V/2022/Bioethic Committe).

2.2. Preparation of *Eucheuma cottonii* extract

E. cottonii was collected from Karimunjawa Beach in Jepara. *E. cottonii* were washed with running water and cut into small pieces. They were dried in an oven at 400°C, and the air-dried *E. cottonii* was then ground into a powder for extraction. 400 g of *E. cottonii* powder was macerated with 2 l of ethanol at 40°C for 3 days. Under vacuum, the supernatant was collected and filtered through Whatman No. 1 filter paper in a Buchner funnel. The filtrate was concentrated by evaporation at 50°C using a rotary vacuum evaporator. The extract was dehydrated under reduced pressure, stored at 0 to 4°C, and used in the experiment.

2.3. Measurement of interleukin-1 (IL-1) level

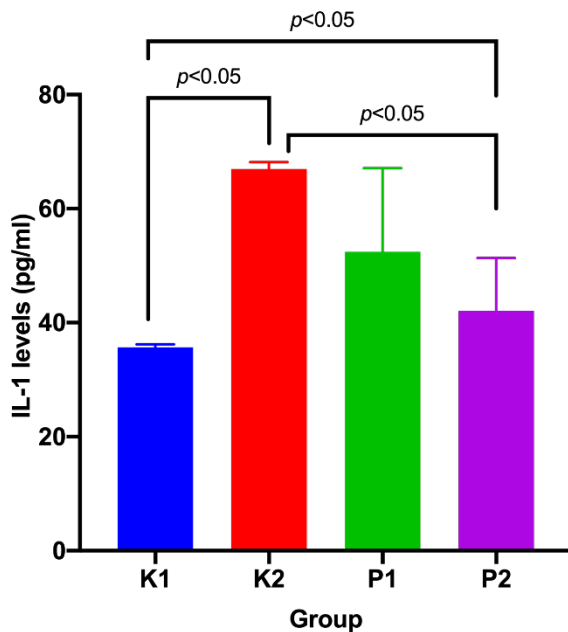
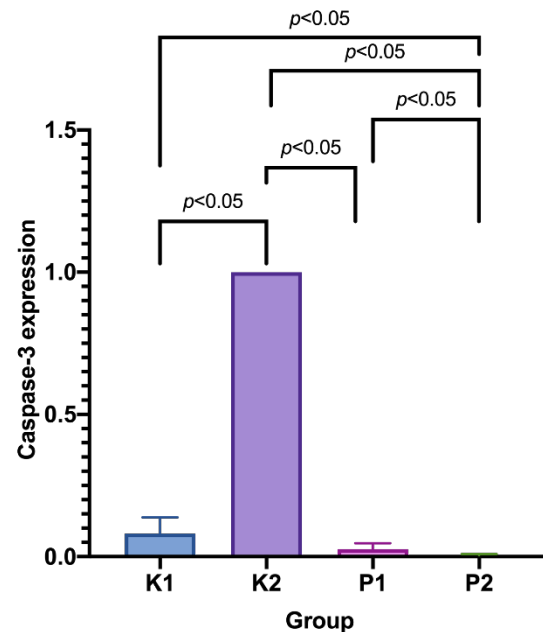
Serum samples were obtained from 20 Wistar rats. RayBio® Rat IL-1 ELISA kit with wash buffer, RDIF, dilution, substrate solution, and standard IL-1 was used as the reagent. 1.0 ml of deionized water was added to the IL-1 Control Kit standard preparation mixture before centrifugation. The sample microplate was prepared, and a series of standard solutions were prepared. 50 µl of RD 1-63 diluents were mounted. Then, 50 l of standard, control, and samples were added, followed by a 2-hour incubation at room temperature. During the incubation period, 2 ml of buffer were prepared. After being incubated for 2 h, they were washed 5x. Then, 100 µl of the IL-1 conjugate was mounted, followed by a second 2-hour incubation at room temperature. After incubation, the washing procedure was repeated 5x. After two hours of incubation, 100 µl of substrate was added, followed by a thirty-minute incubation at room temperature and protection from light. Then, 50 µl of stop solution was added and thoroughly mixed. The results were read for 10 min using a 450 nm ELISA Reader.

2.4. Measurement of caspase-3 expression

RNA was extracted from the liver tissues of each group according to the protocol included in the Real Genomics Total RNA extraction kit. The extracted RNAs were subsequently subjected to reverse transcription polymerase chain reaction using a custom forward primer mixture (Table 1). The information was

Table 1. Gene name, accession number, and primer sequences used in GeXP multiplex analysis in rat

Gene	Primer sequence	
	Forward	Reverse
<i>β-aktin</i>	5'-CATGTACGTTGCTATCCAGGC-3'	5'-CTCCTTAATGTCACGCACGAT-3
Caspase 3	5'-GTGGAAGTACGATGATATGGC-3'	5'-CGCAAAGTGACTGGATGAACC-3'

**Figure 1.** Level of IL-1 in each group.**Figure 2.** Expression of Caspase-3 in each group.

normalized using beta actin (ActB).

2.5 Statistic analysis

The expressions of IL-1 and Caspase 3 were analyzed using Kruskal Wallis followed by Mann Whitney. P values below 0.05 were deemed statistically significant.

3. Results

Male Wistar rats were administered *Eucommia cottonii* extract for 17 days, followed by Borax for 14 days on the fourth day. The results of statistical analysis on the expression levels of IL-1 and Caspase-3 are shown in Figure 1 and 2. IL-1 levels and Caspase-3 expressions were found to be lower in P1 and P2 than in the other control group, according to the study. Kruskal Wallis analysis of IL-1 levels and Caspase-3 expressions revealed a statistically significant difference ($p < 0.05$) between the groups. In addition, a Mann-Whitney test was conducted to determine which group has the greater difference.

The Mann Whitney test revealed that the levels of IL-1 in K2-Groups and P2-Groups were significantly greater than in K1-Groups ($p < 0.05$). P2-Group IL-1 levels were significantly lower than K2-Group IL-1 levels ($p < 0.05$). The difference between P1-G and P2-G IL-1 levels was not statistically significant ($p > 0.05$).

The group administered *E. cottonii* extract at a dose of 1200 mg/200 g had the lowest level of IL-1 (Figure 1).

The Mann Whitney test revealed that Caspase-3 expression in P1-Groups and P2-Groups was significantly lower than in K2-Groups ($p < 0.05$). Caspase-3 expression was significantly lower in P2-groups than in P1-groups ($p < 0.05$). Caspase-3 expression was significantly lower in P2-Groups and K1-Groups compared to K2-Groups ($p < 0.05$). Caspase-3 expression was found to be lowest in the group treated with *Eucommia cottonii* extract at a dose of 1200 mg/200 g (Figure 2).

4. Discussion

Oral administration of *Eucommia cottonii* extract at a dose of 150 mg/kgBW reduced the levels of SGOT and SGPT enzymes and repaired the damage to rat hepatocyte cells caused by sodium nitrite, according to the study's findings (Dewi and Setyawati, 2020). According to phytochemical analyses, *E. cottonii* contains alkaloids, flavonoids, and saponins (Hudaifah et al., 2020). The total flavonoids content of *E. cottonii* extract was approximately 17.78 mg/g (Yanuarti et al., 2017). In addition, carotenoids are present at a concentration of approximately 0.43 mg/g in *E. cottonii* extract (Palawe and Tumonda, 2018).

Borax generates hydroxyl radicals, which activate Kupffer cells to produce proinflammatory

cytokines via the inflammasome signal NLRP3 and the transcription factor NF- κ B (Abul and Andrew, 2016). Among these cytokines is IL-1. In this study, IL-1 levels were significantly lower in the group given 1200 mg/200 g of *E. cottonii* extract than in the control group (K2). Flavonoid is able to inhibit the production of the proinflammatory cytokine IL-1 by inhibiting the NLRP3 inflammasome and caspase-1, there by preventing the conversion of pro-interleukin 1 to interleukin 1 (Lim et al., 2019). Reducing IL-1 receptor kinase 4, which is bound to TLR4 on macrophage cells, is a second mechanism. It will inhibit NF- κ B activity. Consequently, the production of TNF, IL-1, COX-2, and iNOS, which play essential roles in the inflammatory process, decreases (Echave, 2022). Previous research demonstrated that administration of *E. cottonii* extract at a dose of 300 mg/kgBW significantly decreased TNF and NF- κ B expression in mice with asthma (Bakar et al., 2015).

In this study, administration of *E. cottonii* extract at doses of 600 mg/200 g and 1200 mg/200 g significantly reduced caspase-3 expression compared to the control group (K2). The expression of caspase-3 is the final step in both the intrinsic and extrinsic apoptotic pathways. Borax induces the formation of hydroxyl radicals, which influence lipid peroxidation, protein modification, and DNA damage (Kumar and Abbas, 2015). Stress DNA results in the activation of apoptotic cell signalling via the intrinsic pathway. In the presence of DNA stress, the p53 upregulated modulator of apoptosis (PUMA) signal is activated. PUMA then activates Bax in the cytosol and inserts Bax into the mitochondria. The release of proapoptotic molecules such as cytochrome c and apoptosis-inducing factor is triggered by mitochondrial damage (AIF). In hepatocyte cell apoptosis, the cytochrome c complex, APAF-1, forms a Caspase Recruitment Domain (CARD) that activates caspase 9 for procaspase-3 activation, resulting in caspase-3 as the executor caspase (Wong, 2012).

Flavonoids and carotenoids in *E. cottonii* extract exert a direct effect by preventing the formation of reactive free radicals (Arsianti, 2014). In addition, minerals like Fe, Cu, Zn, and Se play a role in the production of antioxidant enzymes like catalase, superoxide dismutase, and glutathione peroxidase (Matanjan et al., 2012).

These enzymes play a role in converting active radicals, such as superoxide and hydroxyl, into less reactive forms (Sue and Kathryn, 2019). This antioxidant's function is to prevent the occurrence of DNA damage in hepatocyte cells caused by borax exposure (Kumar and Pandey, 2013). By preventing DNA damage in hepatocyte, this antioxidant property of *Eucheuma cottonii* extract inhibits the activation of cell

apoptotic signals via the intrinsic pathway. Consequently, there was no formation of apoptosome complexes between cytochrome c and APAF-1. There was also no activation of caspase-9, the caspase activation initiator (Pradhan et al., 2021).

In addition, a decrease in caspase 3 expression can also occur via the extrinsic pathway, as evidenced by the fact that *E. cottonii* extract can also inhibit the production of the proinflammatory cytokine TNF, as demonstrated by previous research. This cytokine binds the cell membrane death receptor and activates caspase-8 to initiate caspase-3 activation (Wong, 2012). Previous research demonstrated that administration of *E. cottonii* extract at a dose of 9 mg/20 g BW could reduce the expression of caspase-8 in trophoblast cells in lead-induced mice using immunohistochemical techniques (Wahyuni and Fajri, 2018). Previous research demonstrated that *E. cottonii* extract inhibits apoptosis via the extrinsic pathway.

5. Conclusion

The administration of *E. cottonii* extract at doses of 600 mg/200 g and 1200 mg/200 g reduces the level of IL-1 and caspase-3 expression in borax-stimulated rat hepatocellular cells. The group administered 1200 mg/200 g *E. cottonii* extract experienced the greatest reduction in IL-1 level and caspase-3 expression.

Conflict of interest

There is no conflict of interest in this publication

References

- Abul, K., Abbas, A., Litchman, A. (2016). *Basic Immunology*. 5th Edition. Philadelphia: Elsevier, pp. 30-35.
- Adinugroho, N. (2103). Pengaruh pemberian boraks dosis bertingkat terhadap perubahan gambaran makroskopis dan mikroskopis hepar selama 28 hari. *Skripsi Universitas Diponegoro*, pp. 1-4. <http://eprints.undip.ac.id/44112/>
- Arsianti, A., Aziza, YA., Kurniasari, K.D., Mandasari, B.K., Masita, R., Zulfa, F.R., et al. Phytochemical test and cytotoxic activity of macroalgae *Eucheuma cottonii* against cervical hela cells. *Pharmacognosy Journal*, 10(5):1012-1017. <http://dx.doi.org/10.5530/pj.2018.5.172>
- Bakar, N.A., Anyanji, V.U., Mustapha, N.M., Lim, S., Mohamed, S. (2015). Seaweed (*Eucheuma cottonii*) reduced inflammation, mucin synthesis, eosinophil infiltration and MMP-9 expressions in asthma-induced rats compared to Loratadine. *Journal of Functional Foods*, 19 (Part A): 710-722. <https://doi.org/10.1016/j.jff.2015.10.011>

- Dewi, I.R.A.P and Setyawati, N.I.W.I. Ida A. Ratih, Ngurah I. Wiratmini, Iriani Setyawati. (2020). Hepatoprotektor rumput laut *Eucheuma cottonii* pada tikus putih (*Rattus norvegicus* L.) jantan yang diinduksi natrium nitrit (NaNO_2). *Metamorfosa : Journal of Biological Sciences*, 7 (2): 87-97. <https://doi.org/10.24843/metamorfosa.2020.v07.i02.p12>
- Echave, J., Otero, P., Garcia-Oliveira, P., Munekata, P.E.S., Pateiro, M., Lorenzo, J.M., Simal-Gandara, J., Prieto, M.A. (2022). Seaweed-derived proteins and peptides: promising marine bioactives. *Antioxidants*, 11 (1): 1-26. <https://doi.org/10.3390/antiox11010176>
- Eva, M. (2020). Uji kadar boraks dan formalin pedagang bakso di wilayah Semarang Tengah Tahun 2020. *Skripsi, Universitas Dian Nuswantoro*. pp. 1-2
- Guyton A. and Hall Je. (2016). *Buku Ajar Fisiologi Kedokteran*. Edisi 12. Jakarta: Penerbit Buku Kedokteran Elsevier, pp. 837-840.
- Hudaifah, I, Mutamimah, D., Utami, A.U. (2020). Komponen Bioaktif dari *Euchemma cottonii*, *Ulva lactuca*, *Halimeda opuntia*, dan *Padina australis*. *Jurnal Ilmu Perikanan dan Kelautan*, 2(2): 63-70. <https://doi.org/10.36526/lemuru.v2i2.1268>
- Kanwal, H. and Sherazi, B.A. (2017). Herbal medicine: Trend of practice, perspective, and limitations in Pakistan. *Asian Pacific Journal of Health Sciences*, 4(4), 6–8. <https://doi.org/10.21276/apjhs.2017.4.4.2>
- Kemenkes RI. (2012). Peraturan Menteri Kesehatan Republik Indonesia Nomor 003/Permenkes/VII/2012 Tentang Bahan Tambahan Makanan. <https://peraturan.go.id/common/dokumen/bn/2012/bn757-2012.pdf>. Accessed on 2 May 2020
- KKP-RI. (2018). Peta Lalu Lintas Rumput Laut Nasional. Kementerian Kelautan dan Perikanan Republik Indonesia. <https://kkp.go.id/bkipm/artikel/8104-peta-lalulintas-rumput-laut-nasional-2018>. Accessed on 2 April 2020
- Kumar, S. and Pandey, A.K. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal*, 2013. 1-16. <https://doi.org/10.1155/2013/162750>
- Kumar, Vinay., Abbas, Abdul K., Aster, Jon C. (2015). *Buku Ajar Patologi Robbins Edisi 9*. Jakarta: Elsevier, pp. 6-18.
- Lim, H., Heo, M.Y., Kim, H.P. (2019). Flavonoids: Broad Spectrum Agents on Chronic Inflammation. *Biomol Ther (Seoul)*. 27(3): 241–253. <https://doi.org/10.4062%2FBiomolther.2019.034>
- Lumbessy, S.Y., Setyowati, D. N., Mukhlis, A., Lestari, D.P., Azhar, F. (2020). Komposisi nutrisi dan kandungan pigmen fotosintesis tiga spesies alga merah (*Rhodophyta sp.*) hasil budidaya. *Journal of Marine Research*, 9(4): 431-438. <https://doi.org/10.14710/jmr.v9i4.28688>
- Matanjun, P., Mohamed, S., Mustapha, N.M. et al. (2009). Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. *Journal of Applied Phycology*, 21, 75–80. <https://doi.org/10.1007/s10811-008-9326-4>
- Neca. H (2021). Pengaruh pemberian ekstrak daun seledri terhadap gambaran mikroskopis sel hepatosit tikus yang diinduksi boraks. *Skripsi Universitas Wahid Hasyim*, Semarang, pp. 33.
- Palawe, J.F.P and Tumonda, S. B. (2018). Analisis kandungan karotenoid sebagai antioksidan dari rumput laut (*Eucheuma cottonii*). *Jurnal Ilmiah Tindalung*, 4(1): 6-9. <http://e-journal.polnustar.ac.id/jit/article/view/126>
- Pradhan, B., Nayak, R., Patra, S., Jit, B.P., Ragusa, A., Jena, M. (2021). Bioactive metabolites from marine algae as potent pharmacophores against oxidative stress-associated human diseases: A comprehensive review. *Molecules*, 26(37): 1-25. <https://doi.org/10.3390/molecules26010037>
- Sue E. Huether, Kathryn L. McCance. (2019). *Buku Ajar Patofisiologi Edisi Ke-6 Vol 1*. Elsevier, pp. 68-70.
- Wahyuni, I and Fajri, L.F. (2018). Pemberian ekstrak rumput laut (*Eucheuma cottonii*) terhadap ekspresi Caspase-8 dan jumlah sel tropoblas pada mencit (*Mus musculus*) bunting sebelum dipapar plumbum (Pb). *Jurnal Sangkareang Mataram*, 4(4): 13-19. <https://sangkareang.org/index.php/Sangkareang/article/view/108/76>
- Wong R. S.Y. (2012). Apoptosis in cancer: From pathogenesis to treatment. *Journal of Experimental & Clinical Cancer Research*, 30 (87): 1-14. <https://jeccr.biomedcentral.com/articles/10.1186/1756-9966-30-87>
- Yanuarti, R., Nurjanah, N., Anwar, E., Hidayat, T. (2017). Profil fenolik dan aktivitas antioksidan dari ekstrak rumput laut *Turbinaria conoides* dan *Eucheuma cottonii*. *Jurnal Pengolahan Hasil Perikanan Indonesia*. 20 (2): 230-237. <http://dx.doi.org/10.17844/jphpi.v20i2.17503>