The Effectiveness Crude Extract of *Cinnamomum Zeylanicum* to the Level of GSH in Mouse Liver

Yuni Ahda[1(✉)], Siska Alicia Farma[1], Yusni Atifah[2], and Jon Efendi[2]

[1] Biology Department Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang, Padang, Indonesia
{ahdayuni,siskaalicia}@fmipa.unp.ac.id

[2] Chemistry Department Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang, Padang, Indonesia

ABSTRACT. *Cinnamomum zeylanicum* is an antioxidant-rich plant, according to numerous research. In this investigation, glutathione (GSH) levels in mouse liver will be compared to those of a crude extract of *Cinnamomum zeylanicum* (*Mus musculus* L.). This research project is an experiment. For a period of four weeks, Thirty mice (*Mus musculus* L.) were divided into three groups: the control group received 75 mg/kg of C. zeylanicum crude extract, while the treatment I and treatment II groups received 150 mg/kg each. The neck dislocation approach resulted in the death of samples on the final day. Every sample is fed at will. The one-way ANOVA test was used to evaluate the data. According to this study, the treatment II group's liver contained the most GSH. When compared to the treatment I and control groups, this level is substantially different ($p < 0.05$). Conclusion: The GSH levels in the liver of Mus musculus are dramatically raised by *Cinnamomum zeylanicum*.

Keywords: Cinnamomum zeylanicum · GSH · liver

1 Introduction

Glutathione (GSH) play an important role in many aspects of metabolisms of live organisms [1–5]. At least it is noted that glutathione is involved in aldehydes, heavy metals, xenobiotics detoxification, disulfides and hydroperoxides reduction, redox signaling, and oxidative protein folding [6–9]. Glutathione is synthesized in cytosol using cysteine, glycine, and glutamate of amino acids [10]. GSH is considered the most important cellular redox buffer and a major defender against oxidative stress [11, 12]. GSSG is produced from GSH by reduction-oxidation processes (oxidized form). The rate of GSH conversion to GSSG is accelerated by oxidative stress, which lowers the GSH/GSSG ratio; thiol-disulfide exchange reaction [13–15], and cell proliferation [16].

The liver is an organ that contains most GSH. The membrane transport of cysteine, cystine, and methionine determines the amount of GSH that is available in hepatocytes [17]. It has been discovered that a methionine deficit causes GSH availability to decline,

M. Fadilah et al. (Eds.): IcoBioSE 2021, ABSR 32, pp. 98–104, 2023.
https://doi.org/10.2991/978-94-6463-166-1_14

particularly in the liver [18]. As a result, by exporting almost all of the produced GSH into plasma and bile, the liver plays a crucial part in preserving the interorgan homeostasis of GSH. The liver should have a large capacity for GSH excretion given its function.

In healthy people, ROS production is balanced with antioxidant production. When there is an increase in ROS production, it will automatically increase antioxidant production. GSH plays a very important role in the ROS–antioxidants balance process. However, when the body is not good in health there will be an increase in ROS levels, resulting in oxidative stress. This will interfere with cellular metabolism and can then damage cell components. If the ROS level is very high above the antioxidant level, a cell can die through apoptosis or necrosis. If the increase of ROS occurs continuously for a long time, prolonged chronic conditions can cause various diseases such as chronic kidney, non-alcoholic hepatitis, and chronic lung obstruction [18].

Many synthetic antioxidants can be consumed to treat diseases due to the increasing level of ROS in cells. However, they cannot be consumed by everyone for several reasons like not being suitable and expensive. On the other hand, many plants are proven antioxidants. One of them is Cinnamomum zeylanicum. In this study, we analysed the effectiveness of C. zeylanicum in decreasing mouse liver GSH.

2 Materials and methods

2.1 *Cinnamomum Zeylanicum* Extraction

A nearby shop sold the Cinnamomum zeylanicum bark, dried. It was confirmed by the taxonomist of the biology department, and then ground into a powder. 100 cc of distilled water were added to the powder mixture in doses of 75 mg and 150 mg.

2.2 *Cinnamomum Zeylanicum* Extraction

In this original study, The Biology Department of Andalas University sold us 30 Wistar rat males (6–8 weeks, 200–250 g). There were acclimated for two weeks in a room that was maintained at a steady temperature of 23–25 °C and had a 12-h cycle of light and darkness. During acclimatization and the duration of the experiment, food and water were freely available to the animals. Then, they were split into three groups at random: the control group (group C), the treatment I group (75 mg/kg of C. zeylanicum crude extract), and the treatment II group (150 mg/kg of C. zeylanicum crude extract). Afterward, Animals were then killed via cervical dislocation. Heart punctures were used to obtain blood samples, which were subsequently centrifuged for 20 min at 10,000 rpm. Serum was then extracted and kept at -80 °C for liver enzyme analyses. The liver tissues were weighted and dissected with care. Additionally, liver samples required for antioxidant enzyme assay were kept in liquid nitrogen.

2.3 Weight Measurements for the Liver and Body

Body weights were measured twice a week and just before the animals were sacrificed. Afterward, using information from the first and preceding week, The experimental groups' weight fluctuations (Weight) were identified. Following the animals were sacrificed, liver weights were also noted.

Table 1. Body Changes and livereight in the experimental periode

Parameter	C	P1	P2
Primary BW	25,55 ± 23,43	25,36 ± 22,6	25,37 ± 25,08
Final BW	29,43 ± 12,53	24,38 ± 21,75[a]	28,47 ± 23,10
⊗ BW	4,18 ± 17,38	1,06 ± 3,70[a]	35,50 ± 18,52
Liver weight	0,57 ± 0,31	0,64 ± 0,58	0,60 ± 0,65

The format for values is mean±SD. C, control; P1, 75 mg/kg, P2, 150 mg/kg

2.4 Calculating the Liver Index

Liver indices were calculated by multiplying the liver weights (g) by the body weights (g) and dividing the result by 100.

2.5 Superoxide Dismutase Determination

According to Kakkar *et al.*, [24], Superoxide Dismutase (SOD) levels were determined by inhibiting the production of nitro-blue tetrazolium, whose optical density was measured at 520 nm. Each SOD unit was defined as the amount of enzyme necessary to reduce the generation rate of chromogen by 50%. Units (U/mg protein) per milligram of protein are used to represent data.

2.6 Analyses of Statistics

Quantitative information was presented as mean SD. One-way analysis of variance (ANOVA) was used for multiple comparisons, followed by the Tukey's posthoc test. When the P-value was less than 0.05, a difference was deemed significant. Data were analyzed done utilizing SPSS.

2.7 Ethical Considerations

Every experiment was carried out using the affirmative vote of Andalas University's animal ethics committee.

3 Result

3.1 Body and Liver Weights Assessments

The first and last weights that were recorded were used to determine weights. FA therapy significantly reduced the ability to acquire weight ($P < 0.001$) (Table 1). This negative effect of FA was reversed by the delivery of 100 mg/kg of CEO (P 0.001). FA furthermore greatly raised the liver's weight ($P < 0.001$); however, 100 mg/kg of CEO treatment significantly decreased this effect ($P < 0.001$).

The format for values is mean ± SD. C, control; P1, 75 mg/kg, P2, 150 mg/kg.

In Fig. 1 shows that the level of GSH increases after being treated with 75 mg/kg and 150 mg/kg of C. zeylanicum for four weeks. This enhancement significantly differs from the control group.

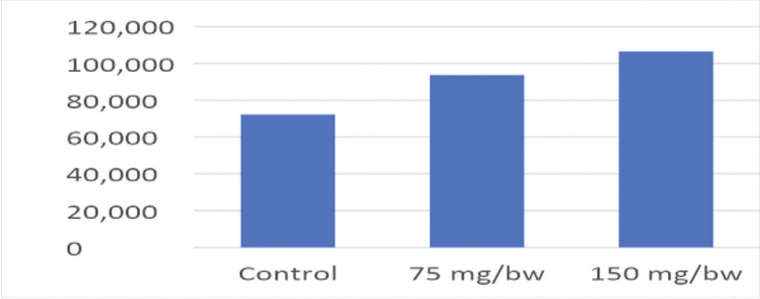

Fig. 1. Level of GSH treated with 75 mg/kg and 150 mg/kg of C. zeylanicum for four weeks. P1 and P2 groups indicate a significant increase in GSH levels after treatment.

3.2 Liver Index Calculation

Utilizing liver weights (g) and body weights (g), liver indices were determined (Table 1). The liver weights in the P1 and P2 groups increased ($P < 0.001$). Crude extract significantly increased body weight at doses of 75 mg/kg and 150 mg/kg ($P = 0.0479$ and $P < 0.001$, respectively).

4 Discussion

Drugs and chemicals like FA are detoxified in large part by the liver [19–21]. The liver's histology and biochemistry have been shown influenced by FA exposure Despite the rats' overall weight reduction, the liver weights of the FA-treated rats (10 mg/kg for four weeks) were found to be substantially higher (Table 1). As a result, the liver index significantly rises in the FA group compared to the control group (Table 1). In each of the three 75 and 150 mg/kg dosages, CEO treatment encouraged animal weight gain while reducing liver weight. However, only in the group receiving CEO at a 100 mg/kg dosage did changes become noticeable. According to studies, FA damages the mitochondria, which reduces the amount of energy that is produced [22, 26]. That could be a reasonable reason for the FA-induced growth inhibition discovered in the present study. The preventive effect of alcohol-based cinnamon extract and carbon tetra-chloride (CCl4) toxicity were assessed in a related investigation by Eidi *et al*., [27]. The outcomes demonstrated that co-treatment with alcoholic extract of cinnamon at concentrations of 0.01, 0.05, and 0.1 g/kg significantly enhanced the liver index by reducing liver weights and increasing body weight.

Additionally, the FA-induced ROS rise would result in an increase in hypereosinophilic and enlarged hepatocytes, parenchymal inflammation, necrosis, and the portal area's disrupted cellular architecture [26]. According to our research, taking FA seriously resulted in significant liver damage. There was a noticeable rise in hypereosinophilic and larger hepatocytes infiltrating inflammatory cells caused widespread localized inflammation in tissue samples from the FA group. Nearly all smaller fields contain necrotic regions. Another indication that FA was harmful is the increased growth of fibrous tissue surrounding the major veins and in the portal regions to the liver tissue.

Additionally, the FA-induced ROS rise would result in an increase in hypereosinophilic and enlarged hepatocytes, parenchymal inflammation, necrosis, and the portal area's disrupted cellular architecture [26]. According to our research, taking FA seriously resulted in significant liver damage. There was a noticeable rise in hypereosinophilic and larger hepatocytes infiltrating inflammatory cells caused widespread localized inflammation in tissue samples from the FA group. Nearly all smaller fields contain necrotic regions. Another indication that FA was toxic to the liver tissue is the increased expansion of fibrous tissue in the portal regions and surrounding the main veins. When 100 mg/kg of CEO was administered, the cellular architectural changes induced by FA in the liver. The CEO100 group's tissue samples showed noticeably fewer hyper-eosinophilic hepatocytes with edema. The portal region and the area around the portal veins were devoid of considerable amounts of fibrous tissue, and there was no longer any localized parenchymal inflammation. Our results are in agreement with study by Eidi *et al*., [27], who discovered that the liver-damaging effects of CCl4 were greatly attenuated by cinnamon alcoholic extract.

5 Conclusion

The use of *C. zeylanicum* has demonstrated that treated mice have higher GSH levels. The treatment of Significantly reducing the large increase in liver weight by 100 mg/kg of CEO (P 0.001). The levels that significantly induce an increase in body weight are 75 mg/kg and 150 mg/kg ($P = 0.0479$ and $P < 0.001$, respectively). To increase the production of antioxidants, it would be advised to ingest the crude extract of C. zeylanicum.

Acknowledgments. The Lembaga Penelitian dan Pengabdian Masyarakat Universitas Negeri Padang, which provided support for this work under contract number 878/UN35.13/LT/ 2021, is acknowledged by the authors.

References

1. D.K. Molina, V.J. DiMaio, Normal organ weights in men: part II- the brain, lungs, liver, spleen, and kidneys, Am J Forensic Med Pathol, 2012, pp. 368–372.
2. D.K. Molina, V.J. DiMaio, Normal organ weights in women: part II. the brain, lungs, liver, spleen, and kidneys, Am J Forensic Med Pathol 2015 vol 36, pp. 182–187.
3. M.N. Gil, D.R. Choi, K.S. Yu, J.H. Jeong, D.H. Bak, D.K. Kim, N.S. Lee, J.H. Lee, Y.G. Jeong, C.S. Na, D.S. Na, K.H. Ryu, S.Y. Han, Rhus verniciflua stokes attenuates cholestatic liver cirrhosis-induced interstitial fibrosis via Smad3 down-regulation and Smad7 up-regulation, Anat Cell Biology, 2016, pp. 189–198.
4. A. Bhattacharya, P. Dhar, R.D. Mehra, Preliminary morphological and biochemical changes in rat liver following postnatal exposure to sodium arsenite, Anat Cell Biol, 2012, pp. 229–240.
5. N. Saowakon, P. Ngernsoungnern, P. Watcharavitoon, A. Ngernsoungnern, R. Kosanlavit, Formaldehyde exposure in gross anatomy laboratory of Suranaree University of Technology: a comparison of area and personal sampling, Environ Sci Pollut Res Int, 2015, pp. 19002-190012.

6. M. Bunyavaree, P. Kasemsarn, and W. Boonchai, Cosmetic preservative labelling on the Thai market, Contact Dermatitis, 2016, pp. 217–221.
7. L.T. Ou, Enhanced degradation of the volatile fumigant-nemati- cides 1,3-d and methyl bromide in soil, J Nematol, 1998, pp. 56–64.
8. S.E. O'Quinn, C.B. Kennedy, Contact dermatitis due to formaldehyde in clothing textiles, iJAMA 1965, pp. 593–596.
9. S. Viegas, C. Ladeira, C. Nunes, J. Malta-Vacas, M. Gomes, M. Brito, P. Mendonca, J. Prista, Genotoxic effects in occupational exposure to formaldehyde: a study in anatomy and pathology laboratories and formaldehyde resins production, J Occup Med Toxicol, 2010.
10. D. Kotzias, O. Geiss, S. Tirendi, J. Barrero-Moreno, V. Reina, A. Gotti, G. Cimino-Reale, B. Casati, E. Marafante, D. Sarigiannis. Exposure to multiple air contaminants in public buildings, schools and kindergartens: The European Indoor Air Monitoring and Exposure Assessment Study (AIRMEX) Study, Fresen Environ Bull, 2009, 670–681.
11. S. Teng, K. Beard, J. Pourahmad, M. Moridani, E. Easson, R. Poon, P.J. O'Brien, The formaldehyde metabolic detoxification enzyme systems and molecular cytotoxic mechanism in isolated rat hepatocytes, Chem Biol Interact, 2001, pp. 285–296.
12. J.R. Beall, A.G. Ulsamer, Formaldehyde and hepatotoxicity: a re-view, J Toxicol Environ Health, 1984, pp. 1–21.
13. T. Matsuoka, A. Takaki, H. Ohtaki, S. Shioda, Early changes to oxidative stress levels following exposure to formaldehyde in ICR mice, J Toxicol Sci, 2010, pp. 721–730.
14. G.D. Noudeh, F. Sharififar, A.D. Noodeh, M.H. Moshafi, M.A. Afzadi, E. Behravan, M. Aref, R. Sakhtianchi, Antitumor and antibacterial activity of four fractions from Heracleum persicum Desf. and Cinnamomum zeylanicum Blume, J Med Plants Res 2010 vol 4, pp. 2176–2180.
15. K. Sambaiah, K. Srinivasan, Effect of cumin, cinnamon, ginger, mustard and tamarind in induced hypercholesterolemic rats, Nahrung, 1991, pp. 47–51.
16. A. Eidi, P. Mortazavi, M. Bazargan, J. Zaringhalam, Hepatoprotective activity of cinnamon ethanolic extract against CCI4-induced liver injury in rats, EXCLI J, 2012, pp. 495–507.
17. D. Gunawardena, N. Karunaweera, S. Lee, F.V.D. Kooy, D.G. Harman, R. Raju, L. Bennett, E. Gyengesi, N.J. Sucher, G. Münch, Anti-inflammatory activity of cinnamon (C. zeylanicum and C. cassia) extracts: identification of E-cinnamaldehyde and o-methoxy cinnamaldehyde as the most potent bioactive compounds, Food Funct, 2015, p. 910 - 919.
18. S.A. Maisonneune, European Pharmacopoeia, Vol. 3. Saint-Ruff, Maisonneuve, 1975.
19. J. Malik, K. Munjal, R. Deshmukh, Attenuating effect of standardized lyophilized Cinnamomum zeylanicum bark extract against streptozotocin-induced experimental dementia of Alzheimer's type, J Basic Clin Physiol Pharmacol, 2015, pp. 275–285.
20. S. Jain, T. Sangma, S.K. Shukla, P.K. Mediratta, Effect of Cinnamonmum zeylanicum extract on scopolamine-induced cognitive impairment and oxidative stress in rats, Nutr Neurosci, 2015, pp. 210- 216.
21. A. Zeenat, R. Claire, A.H. Neo AH, C.W. CW, J.S. Lim, C.C. Aw, E.R. Browne, D.J. Pemberton, Alternative method of oral dosing for rats, J Am Assoc Lab Anim Sci, 2010, pp. 335–343.
22. L.F. Van Pelt, Ketamine and xylazine for surgical anesthesia in rats, J Am Vet Med Assoc 1977, pp. 842–844.
23. P. Kakkar, B. Das, P.N. Viswanathan, A modified spectrophotometric assay of superoxide dismutase, Indian J Biochem Biophys, 1984, pp. 130–132.
24. W. Wasowicz, J. Neve, A. Peretz, Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage, Clin Chem, 1993, pp. 2522–2526.
25. D.E. Paglia, W.N. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, J Lab Clin Med, 1967, pp. 158–169.

26. A.K. Sinha, Colorimetric assay of catalase, Anal Biochem, 1972, pp. 389–394.
27. C. Peralta, G. Hotter, D. Closa, N. Prats, C. Xaus C, E. Gelpí, J. Roselló- Catafau, The protective role of adenosine in inducing nitric oxide synthesis in rat liver ischemia preconditioning is mediated by activation of adenosine A2 receptors, Hepatology 1999, pp.126- 132.
28. S. Hortelano, A.M. Genaro, L. Bosca, Phorbol esters induce nitric oxide synthase activity in rat hepatocytes, Antagonism with the induction elicited by lipopolysaccharide, J Biol Chem, 1992, pp. 24937–24940.