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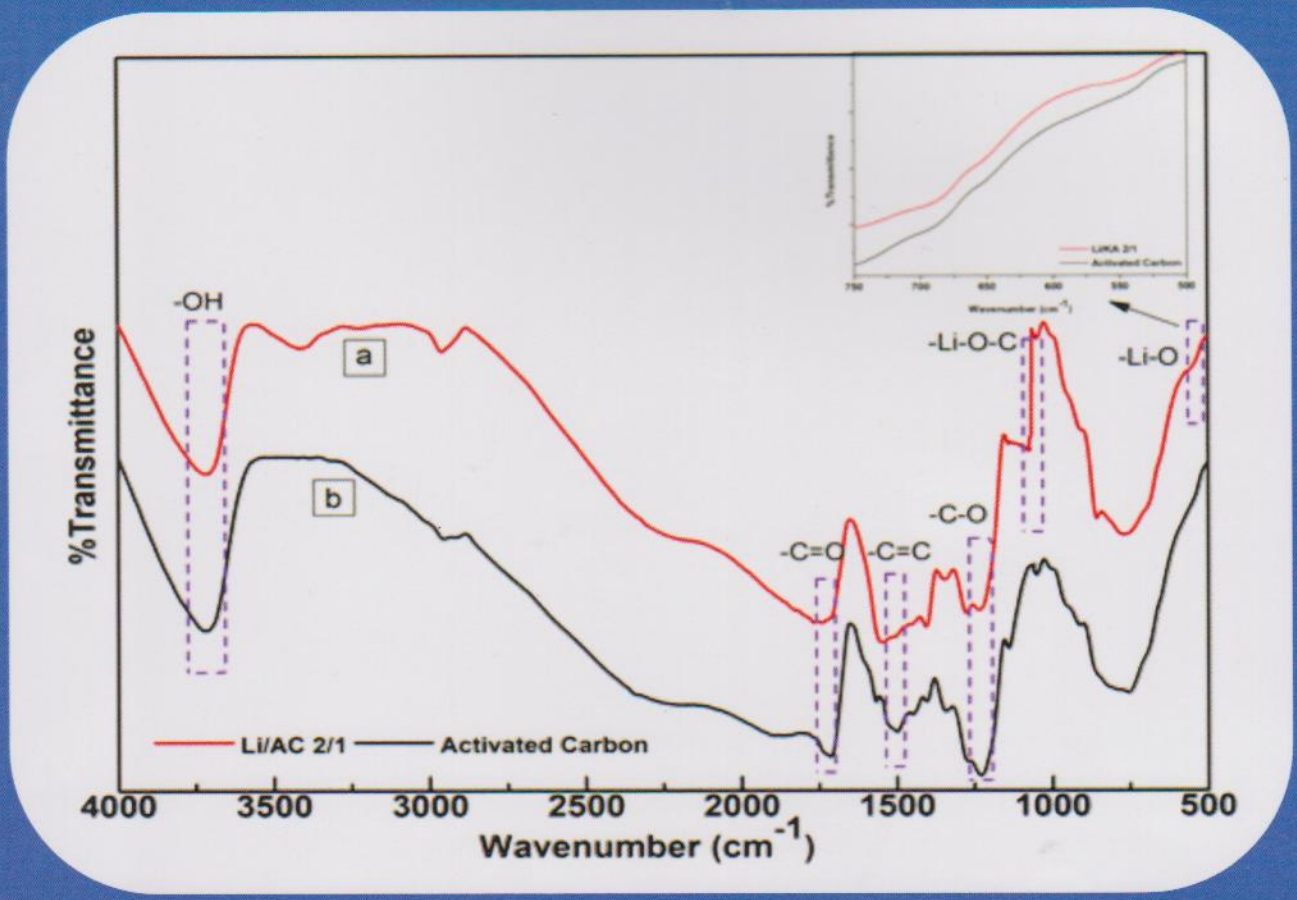

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Metrics

	2019	2020	2021
Scopus CiteScore	0.1	0.3	0.7
Copernicus ICV	99.19	100	
Google Scholar h-index	10	12	13
Acceptance rate	72%	46%	40%

Most read articles in last issue

Preparation and Antimicrobial Activity Analysis of Organic Soap Bar Containing Gnetum gnemon Peel Extract

Sri Handayani, Indyah Sulistyo Arty, Cornelia Budimarwanti, Karim Theresih, Evy Yulianti, Melati Khairuddean

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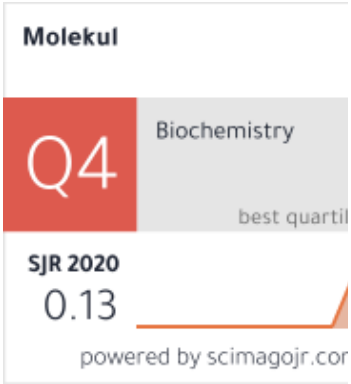

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MOLEKUL is a scientific journal in chemistry, **focus on** the regional resources, environment or natural products of Indonesia and nearby tropical country.

The scope of this journal including :

- Theoretical chemistry and environmental chemistry study
- Material synthesis for energy and environmental
- Isolation, purification and modification of biomolecules, including their applications
- Fabrication, development and validation of analytical methods

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History

Molekul has been established since 2006 with volume 1 no 1 (November 2006). The name of the birth of certificate from the Universitas Jenderal Soedirman was "Jurnal Ilmiah Kimia "Molekul". Jurnal Ilmiah Kimia mean Scientific Journal in Chemistry. The journal name in the quotes was Molekul. Furthermore, the journal cover used the name MOLEKUL and a small Jurnal Ilmiah Kimia. The running journal name in all articles is Molekul. Print ISSN name was Molekul, electronic ISSN was Molekul: Jurnal Ilmiah Kimia. The name of the journal in the National Accreditation certificate was Jurnal Ilmiah Kimia Molekul. The final name of the journal should be only **Molekul**.

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Molekul

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Articles

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Anti Inflammatory Test of *Centella asiatica* Extract on Cadmium Induced Rats

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ABSTRACT. Cadmium is a toxic heavy metal which pollute the environment parallel with human activities in such industries like textiles and weldings. The presence of cadmium in human body caused liver inflammation. This study was aimed to know the anti inflammatory effects of *Centella asiatica* extract on cadmium induced rats. This research used an experimental study applying a post test only control group design. Twenty four rats were devided into six groups with four replications, i.e group of healthy control (C1), negative control ($CdSO_4$-induced rats) at the dosage of 56 mg/kgbw for 10 days. Meanwhile (C3-C6 treatments) had dosage of 100, 200, 300 and 400 mg/kgbw The blood Cd content, GST, GSH, TNF- α and COX-2 were measured following the administration of C. *asiatica* 14 days. and analyzed by ANOVA test. Statistical analysis will be continued by the Duncan test when there was a significance difference at 5%. The result showed that administration of C.*asiatica* extract was able to neutralize cadmium, as well as restoring the inflammatory level. The conclusion of this research that C. *asiatica* extract at dosage of 200 mg/kgbw was the most effective dosage to decrease Cd level, TNF-α and COX-2 levels but increase GST and GSH level

Key words : anti inflammatory, Cd, *Centella asiatica*, GST, COX-2

INTRODUCTION

The heavy metal cadmium (Cd) is a dangerous compound since it can pollute the environment and cause health problems for humans. Increasement of Cd pollutant is parallel to the increasement of human activities in such industries as the textile, battery, paint, plastics, welding, fertilizers and pesticides. (Bernhoft, 2013., Chunhabundit, 2016). Exposure of Cd to human causes some damages on liver kidney, lung, emphysema and so hypertension; and liver inflammation (Fatima, Raza, Hadi, Nigam, & Mahdi, 2019., Zhang and Reynold, 2019., Genchi, Sinicropi, Lauria, & Carocci, 2020).

Once the Cadmium enters human body will bind with metallothionein protein in the liver to form a Cd-MT complex which is stable bond and is difficult to release. The Cd-MT complex triggers the formation of free radicals and lipid peroxidation and caused inflammation in the liver (Sarkar, Ravindran, & Krishnamurthy, 2013., Rani, Kumar, Lal, & Pant, 2014., Das & Al-Naemi, 2019). Glutathione-S Transferase (GST) in liver helps to detoxify toxical compounds enters the body. Decreasment of total GST as well as Glutathione (GSH) a precursor of GST are the main characteristics of liver failure since they will lowering down the main function of liver, leading to inactivation of the GST. Damage or failure on the hepatic cell membrane results in the induction of intracellular liver enzymes into the blood vessels, such as the Alanine Transferase (ALT) and Aspartat Transferase (AST) enzymes. The level of these two enzymes in the blood are therefore increased (Nikniaz, Nikniaz, Tabrizi, Sadeghi-Bazargani, & Farahbakhsh, 2018). One clinical study showed that environmental Cd exposure was either related with hepatic necro-inflammation, non-alcoholic fatty liver disease (NAFLD), and non-alcoholic steato hepatitis (NASH) in men or hepatic necro-inflammation in women (Choi et al., 2016). In order to minimizing the effect of Cd in the patient'body, medical doctor might give chemical chelator called Dimercaprol, but unfortunately this medicament does not able to remove complex Cd-MT bonds and also couse hypertension. In addition Cd-MT generate Reactive Oxygen Species and simultaneously decrease endogenous antioxidant such as Super Oxide Dismutase (SOD), Glutathione Peroxidase (GPx), Catalase (CAT), Glutathione, ascorbic acid and tocopherol which are known oxidative stress (Bernhoft, 2013., Hernayanti, Santoso, & Lestari, 2018).

In the mean times, application of exogenous antioxidants have been being intensively in protecting the cell's damages as well as eliminating free radicals which caused by oxidative stress. Some sources like

vegetables, fruits and medicinal plants have been explored in many laboratories; and becoming candidates in preventing cells from oxidative stress, and simultaneously promoting the costumer's health due to its phenolic compounds. Later the phenolic compounds was also known to play important rule in scavenging and neutralizing free radicals through its oxide-reduction properties. (Symonowicz & Kolanek, 2012)

Centella asiatica a perennial herb has been used for treating several disorders, such as insanity, asthma, leprosy, ulcers and eczema and for wound healing. So far about 20 species related to *C. asiatica* grown in most parts of the tropic or wet pantropical areas such as rice paddies, and also in rocky, higher elevations. The *C. asiatica* is a tasteless, odourless plant that thrives in and around water. It also has small fan-shaped green leaves with white or light purple-to-pink or white flowers and bears small oval fruit. The whole plant is used for medicinal purposes (Chong, Aziz, Jhala, & Thaker, 2014). In Ayurvedic medicine, Centella is effectively used in the treatment of inflammation, anaemia, asthma, blood disorders, bronchitis, fever, urinary discharge and splenomegaly (Singh, Singh, Gupta, Solanki, & Nema, 2012). The aqueous extract of Centella possesses antioxidant, cognitive enhancing, and antiepileptic properties. The anti oxidative property of Centella may play an important role in reducing the activity of reactive oxygen species (ROS) in the body system (Hamid, Shah, Muse, & Mohamed, 2002). The herb is also taken as a tonic for poor digestion and rheumatism; the latter suggest it may have anti-inflammatory effects. Anti-inflammatory effects which associated with glycosides and terpenes compounds (Hashim, Sidek, Helme, Helan, & Sabery, 2011).

C. asiatica contains active constituents, the constituent like triterpenoid, saponins,flavonoids, tannins including asiaticoside, centelloside. Madecassoside and asiatic acid are the important ones. Asiaticoside is the most abundant of triterpene glycoside in water extraction and it is transformed into asiatic acid in vivo by hydrolysis (Singh, Singh, Gupta, Solanki, & Nema, 2012., Jenwitheesuk, Rojsanga, Chowchuen, & Surakunprapha, 2018). These two compounds of asiaticoside and asiatic acid have been reported to have protective effects against kidney injury following an induced sepsis, more likely associated with the inhibition of IL-6 and TNF α in serum and the inducible Nitric Oxide Synthase (iNOS) enzyme in kidney tissues. Madecassoside may be potential as anti-inflammatory agents via the inhibition of iNOS, cyclooxygenase-2 (COX-2), interleukins (IL-6, IL-1β) and cytokine tumor necrosis factor (TNF-α) expression through the down-regulation of NF-κB activation (Qiu et al., 2015; Yasurin, Sriariyanun, & Phusantisampan, 2016., Sasmita, Ling, Voon, Koh, & Wong, 2018). In addition, *C.asiatica* contains also other components, including volatile oils, flavonoids, tannins, phytosterol, amino acid and sugar.

The aim of the study was to determine the effect of *C. asiatica* and the most effective dose of *C. asiatica* in reducing cadmium levels and TNF-α, COX-2 and simultaneously increasing GST and GSH.

EXPERIMENTAL SECTION

The current study was a true experimental one with a post test only control group design. A total of 24 Wistar rat samples were randomly selected and divided into six groups i.e. group of healthy control (C1), negative control which was induced by CdSO4 at the dosage of 56 mg/kg for 14 days and treatment (C3-C6). The treatment groups were given *C. asiatica* extract at the following dosages of 100, 200, 300 and 400 mg/kg of. *C. asiatica*. The study was approved under the ethical clearance from The Moewardi General Hospital No. 298/II/HREC/2020.

Preparation of Experimental Animals

Twenty four Male Wistar rats strain at the age 2-3 months, weight of 180-200 g, obtained from The Integrated Research and Testing Laboratory IV UGM were selected and adapted for one week. The treated rats were fed pellet AD II and provided for water *ad libitum*. Plastic box for rats cage and the base of cage were coated ± 2 cm of straws. The base straw were replace for 2 days. The cage temperature, air circulation and room lighting was set up as a controlable environment

Making of $CdSO_4$ Solution

The LD_{50} $CdSO_4$ dosage is 280 mg/kg human body weight, this amount then converted to 56 mg/gbw of rats. The study applied the dose of 25% of LD_{50} $CdSO_4$ which was equal to 14 mg/gbw rats. The $CdSO_4$ was dissolved into 48 mL aquadest. Each rats was given 2 mL $CdSO_4$ solution orally for 10 days.

Making of *C. asiatica Extract*

Aqueous *C. asiatica* was prepared by maceration method in the following step. 10 g dried-leaves of *C. asiatica* was added by 400 mL ethanol 96 % and left for 24 hours. Re-maceration was done three times in 24 hours, repeatedly. After 3x 24 hours macerate removed to *Vacum Rotary Evaporator* to obtain thick extract. A thick extract be weighed to make the dosage of 100, 200, 300 and 400 mg/kg respectively. The conversion dosage were 20, 40, 60 and 80/200 g/bw. The thick extract was dissolved in 10 mL aquadest. The administration of thick extract for 14 days according to the treatment dose, after rats induced by $CdSO_4$. Each treated rats (C3-C6) was given 2 mL extract orally.

Treatment of Animals

A capillary hematocrite pipette was used to take the rat's blood on the vein orbitalis plexus following a treatment of $CdSO_4$ solution for 10 days and

administration by C. *asiatica* extract (C3-C6), rats healthy control (C1) and Cadmium- induced rats (C2. Before blood withdrawn the rats anesthetized with ether. Five mL blood was collected on Eppendorf tube of these volume 2 mL was used for blood's Cd examination and the rest (3 mL of blood) for measuring GST, GSH, TNF-α and COX-2 levels. Blood cadmium is measured by AAS machine at 228.6 nm wavelength and a strong current of $3.5mA^0$. Further the rest of blood (3mL) centrifuge for 10 minutes with a speed of 6,000 rpm then serum is separated from the blood. A quantitative GSH and GST, TNF-α and COX-2 levels in serum were measured by ELISA method (BT Laboratories, Shanghai China), according to the instructions available for suppliers.

Data Analysis

Data were analyzed by Analysis of Variance (ANOVA) at significant rate 95%, followed by Duncan test to determine the differences between each group and the most effective dose of C. *asiatica* extract

RESULT AND DISCUSSION

Result of blood Cd level as shown in **Figure1.** The highest of Cd level containing in the rats blood was shown in animals belong to C2 group, where the treated rats were given the lowest concentration of C.*asiatica* extract but simultaneously given the $CdSO_4$. Data analysis among the healthy control, positive control and treated groups were significantly different. These data show that Cadmium enters the rats body and binds to metalothionein and further it generates oxidative stress which occurs when the generation of free radicals or reactive oxygen species (ROS) such as $^1O_2$, O_2^- and OH^- exceed the antioxidant capacity of a biological system (Bernhoft, 2013., Prabu & Bashir, 2014). Excess number of free radicals and ROS attack biological molecules like lipids, proteins and nucleic acids which undergoes tissue or cellular injury and lipid peroxidation especially in liver organ. In normal condition the Cd enters the liver will be neutralized by Glutathione S- Transferase (GST) and glutathione (GSH) which role as substrate of this enzyme. The origin GSH was called cysteine supplied from protein synthesis in the liver. If the number of GSH in liver are sufficient the GST can start detoxifying Cd. Damaging of liver organ however caused by free radical resulted from insufficient amount of GSH production and inactivated the GST (Satarug, Garrett, Sens, & Sens, 2010., Bernhoft, 2013., Llavanera et al., 2020). Intoxication of Cd may cause inflammation where pro-inflammatory condition have been reported as a result of production and upregulation of IL-6, TNF-α and IL-1β in vivo and in vitro. The TNF-α is a cytokine produced by activated macrophages in response to injurious. IL-1 β is expressed that play a role in the activation of NF-κB the gene of proinflammatory cytokine and chemokine (Das & Al-Naemi, 2019).

Current study noted that administration of C. *asiatica* extract for 14 consecutive days resulted in improvements in the liver function of those treated group. In this group, the Cd level in the blood shown to normal level. The data analysis showed very significant differences between control and treatment groups. The component called madecassosida of C. *asiatica* was reported to be able to protect the liver (Sivakumar, Sadiq, & Bharathi, 2018). Madecassosida is a type of quercetin derivate of flavonoid which are able to chelate metal ions such as Cd in its 3 'and 4' phenolic groups. Quercetin compounds are able to chelate metal ions, such as cobalt (Co^{2+}) and aluminum (Al^{3+}) and Plumbum (Pb^{2+}) (Symonowicz & Kolanek, 2012). Alternatively the madecassosida act as a radical scavenger by donating the H^+ to free radical so it becomes neutral and breaks the lipid peroxidation chain in liver. Finally this condition leads to improve the liver damage (Salim, Adenan, Amid, Jauri, & Sued, 2013., Qiu et al., 2015., Zhao et al., 2014).

The antioxidant activity of aquaous extract (AE) of C. *asiatica* in vitro was evaluated by its ability to scavenge DPPH free radicals. The radical scavenging activity of the compounds can be measured by the decolorizing effect following the trapping of the unpaired electrons of DPPH. The AE showed a high antioxidant activity, with an IC_{50} value of 31.25 µg/mL. Ascorbic acid and butylated hydroxytoluene (BHT) produced IC_{50} values of 2.50 µg/mL and 7.58 µg/mL, respectively. Based on previous data, it is possible that the powerful antioxidant activity of polar extracts is due to the presence of substances with free hydroxyls. The antioxidant activity of Centella (84%) was compared to grape seed extract (83%) and Vitamin C (88%). In addition the hexane fraction of CA showed potential activity with chelating activity to $FeSO_4$ at IC_{50}= 0.090 and the ethyl acetate fraction of EC had IC_{50}= 0.120 mg/mL (Rahman et al., 2013).

Hashim et al., 2011 state that C. *asiatica* has antioxidant activity 84% compared to grape seed extract (83%) and Vitamin C (88%). The lipolytic activity of Centella indicated by the release of glycerol (115.9 µmol/L) at 0.02% concentration.

Gluthatione Level (GSH)

Result of GSH level as shown in **Figure 2.** C. *asiatica* extracts all dosages increase the GSH level by donating the H^+ of quersetin to neutralize the ROS by inhibiting lipid peroxidation and improving liver's damage. Extract of C. *asiatica* consists also amino acid which synthesis GSH thus, and finally the liver can restart producing GSH (Deb, Puthanveetil, & Sakharkar, 2018., Sivakumar et al., 2018). This case followed by increasing of GST level.

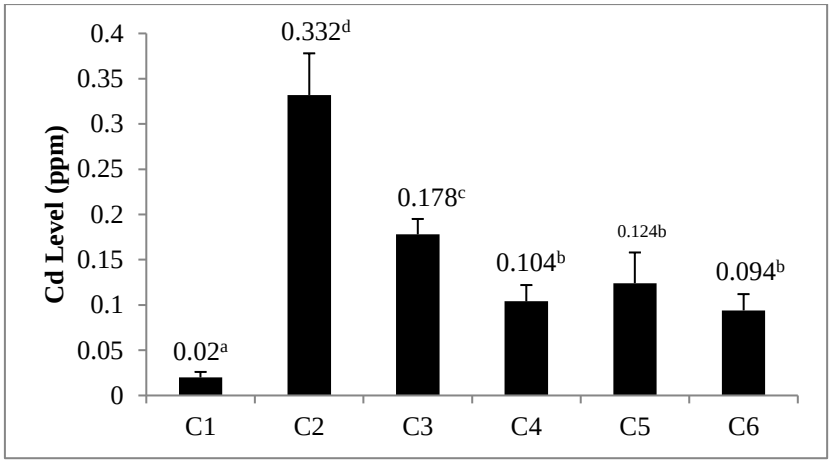

Figure 1. Blood Cd level in control and treatment groups. C1 (healthy control), C2 (positive control induced by $CdSO_4$), C3 (treatment with C. *asiatica* extract 100 mg/kgbw, C4 (treatment with C. *asiatica* extract 200 mg/kg), C5 (treatment with C. *asiatica* extract 300 mg/kgbw) and C6 (treatment with C. *asiatica* extract 400 mg/kgbw).

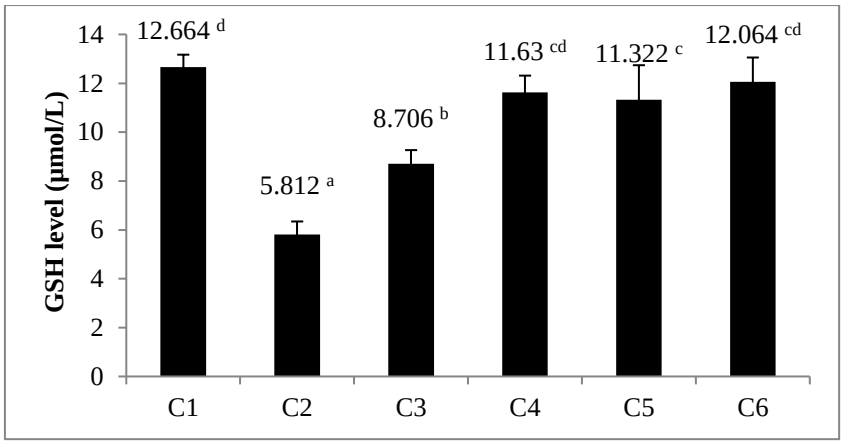

Figure 2. GSH level in control and treatment groups. C1 (healthy control), C2 (positive control induced by $CdSO_4$), C3 (treatment with C. *asiatica* extract 100 mg/kg, C4 (treatment with C. *asiatica* extract 200 mg/kgbw), C5 (treatment with C. *asiatica* extract 300 mg/kgbw) and C6 (treatment with C. *asiatica* extract 400 mg/kgbw).

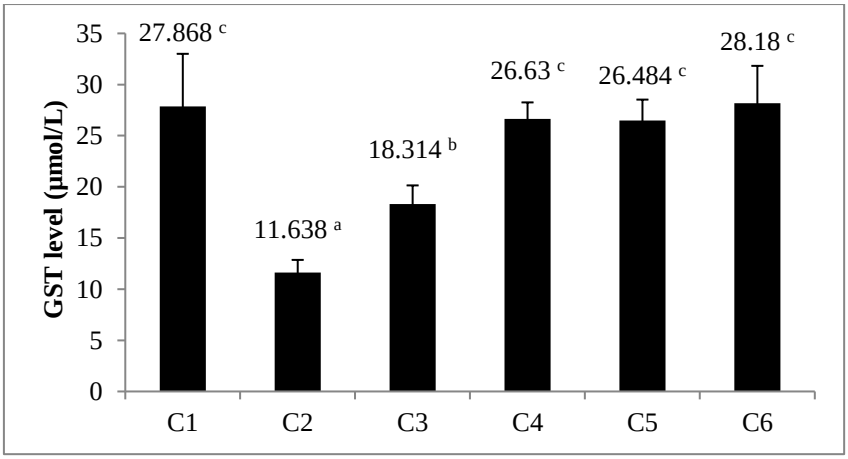

Figure 3. GST levels in control and treatment groups. C1 (healthy control), C2 (positive control induced by $CdSO_4$), C3 (treatment with C. *asiatica* extract 100 mg/kgbw, C4 (treatment with C. *asiatica* extract 200 mg/kgbw), C5 (treatment with C. *asiatica* extract 300 mg/kgbw) and C6 (treatment with C. *asiatica* extract 400 mg/kgbw).

Glutathione S- Transferase

The GST enzyme takes important roles in catalyzing the conjugation of hydrophobic toxicant and electrophilic compound such as Cd. The GST together with the GSH, a conjugation substrate of GSH-Cd breaks down enzyme and acetylation lead to form of hydrophilic N-acetyl cysteine (mercapturic acid) the Cd element is therefore easily to be excreted in urine (Okat, 2018). The current study show that all of dosages C. *asiatica* were able to decrease Cd level but simultaneously increase the GSH and GST level. It happens because the C. *asiatica* extract chelates such heavy metal like $FeSO_4$ and Lead acetate (Ponnusamy, Mohan, & Nagaraja, 2008). C. *asiatica* extract inhibited the proliferation of a malignant HepG2 cell line through apoptosis or programmed cell death so that it has potential to be used as anti liver cancer (Hussin, Eshkoor, Rahmat, Othman, & Akim, 2014).

Madecassosida may also act as a radical scavenger by donating its H^+ to free radical leads to aneutral condition to break lipid peroxidation chain in liver and finally improve the hepar's damage (Yasurin et al., 2016). Result of GST level as shown in **Figure 3**.

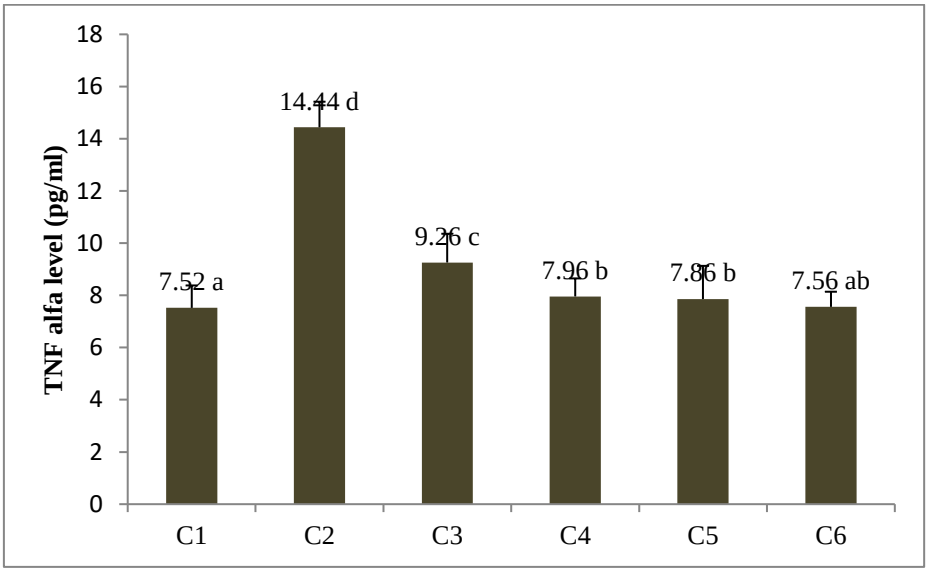

Figure 4. The average level TNF-α of in control group and treatment with C. *asiatica*. C1 (healthy control), C2 (positive control induced by $CdSO_4$), C3 (treatment with C. *asiatica* extract 100 mg/kgbw, C4 (treatment with C. *asiatica* extract 200 mg/kgbw), C5 (treatment with C. *asiatica* extract 300 mg/kgbw) and C6 (treatment with C. *asiatica* extract 400 mg/kgbw).

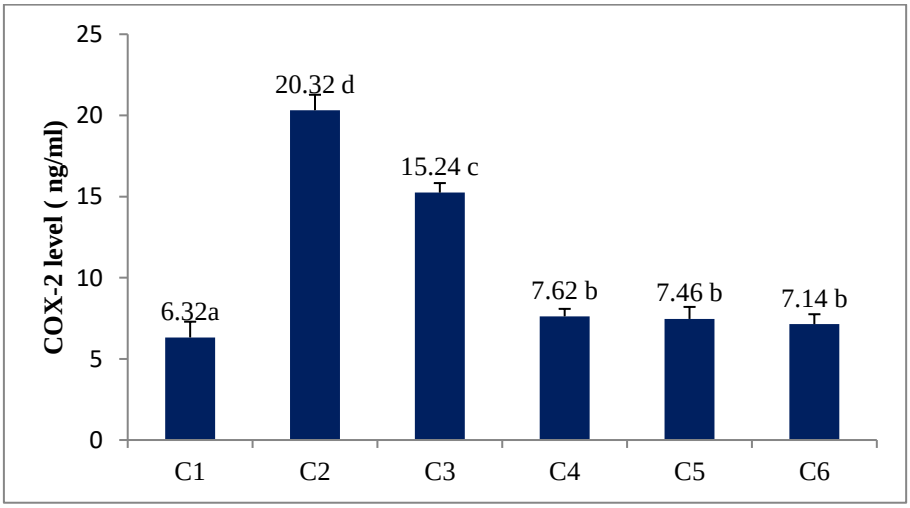

Figure 5. The average COX-2 levels in control group and treatment with C. *asiatica*. C1 (healthy control), C2 (positive control induced by $CdSO_4$), C3 (treatment with C. *asiatica* extract 100 mg/kgbw, C4 (treatment with C. *asiatica* extract 200 mg/kgbw), C5 (treatment with C. *asiatica* extract 300 mg/kgbw) and C6 (treatment with C. *asiatica* extract 400 mg/kgbw).

Tumor Necrosis Factor-α (TNF-α) Level

The average result of TNF-α level as shown in **Figure 4. Figure 4** showed that the highest level of TNF-α level reflected in the C2 treatment (Cd was induced). The higher the TNF- α the more Cd-MT bond formed and causes liver damage due to inflammatory response. The inflammatory status begins when NF-κB is activated which generated by macrophage in the liver. The NF-κB is responsible for the release of cytokine, such an important component for anti-inflammatory activity. Pro-inflammatory cytokines, including IL-4, IL-6, IL-1β, and TNF-α, commonly contribute to the regulation of inflammation and immune responses. TNF-α, a cytokine involved in systemic inflammation stimulates the acute phase reaction. The primary role of TNF- α an endogenous pyrogen, lies in the regulation of immune cells by inducing fever, sepsis and apoptotic cell death. This protein is chiefly produced by activated macrophages although it can be produced by other cell types as well (Somboonwong, Kankaisre, Tantisira, & Tantisira, 2012., Park et al., 2017).

Administration of C. *asiatica* for 14 days decreases the TNF-α level. because the asiaticoside, such a component of C. *asiatica* plays important role in anti-inflammatory effect by lowering down the NF-κB signaling pathway. It is also contributing to the reduction of iNOS expression which finally decrease the level of TNF-α (Sasmita et al., 2018., Wan, Gong, Jiang, Zhang, & Zhang, 2013). Whereas the, madecassic acid was reported also to have anti-inflammatory activity through the down regulation of iNOS expression, IL-1β, and IL-6 release in RAW264.7 macrophage cells (Won et al., 2010).

Cyclooxygenase-2 (COX-2)

The average result of COX-2 level as shown in **Figure 5**. **Figure 5** showed the highest level of COX-2 level was shown by the C2(treatment Cd induced). The higher the cadmium level, the more COX-2 level since the Cd component lowering the mRNA expression of COX-2. The last compound (COX-2) is an enzyme which highly inducible by pro-inflammatory cytokines, tumor promoters, mitogens, and growth factors in a variety of cell types, including monocytes, leads to an increase the release of prostalglandin. COX-2 is the major contributor to prostanoid synthesis as inflammation progresses in rat induced by cadmium (Park, Hong, & Jang, 2012)

Following to the administration of C. *asiatica* extract for 14 days the level of COX-2 was decreased. Cho et al, 2020 state that methanolic extraction of C. *asiatica* extract suppressed iNOS and inhibited the COX-2 levels by inhibiting transforming growth factor-β-activated kinase 1 (TAK1) phosphorylation and IL-1 receptor-associated kinase (IRAK1) and further degradating in the suppression of nuclear factor kB. Asiaticoside contribute to the reduced of TNF-α, IL-6, and IL-1β level. *Monocyte Chemoattractant Protein-1* (MCP-1) regulated inflammation response significantly suppressed nitric oxide production and iNOS expression in RAW 264.7 macrophages. Prostaglandin E_2 production was alleviated by MCA via the downregulation of cyclooxygenase-2 (Hafiz et al., 2020., Qiu et al., 2015). The asiaticoside is potentially to be applied for inhibiting prostaglandins, a group of proinflammatory signaling molecules which mainly due to inhibition of key enzymes involved in prostaglandin biosynthesis (lipoxygenase, phospholipase and cyclooxygenase) (Won et al., 2010).

The current data showed, administration all dosages of C *asiatica* were able to decrease Cd level, TNFα, COX-2 levels but simultaneously increase the GSH and GST levels.The dosage of 200mg/kgbw, 300mg/kgbw, 400mg/kgbw showed a better effect than 100 mg/kgbw. The dosage of 200 mg/kgbw is sufficient enough to recover liver's damage. So that dosage of C. *asiatica* 200 mg/kgbw was the minimal effective dose for chelating agent of Cd and may be used to overcome inflammation in Cd poisoning.

CONCLUSION

The administration of C. *asiatica* extract can protect liver of rat induced by $CdSO_4$. This result was indicated by the decrease of blood Cd level, TNF α, COX-2 levels but increase the GSH and GST levels. The recommended dose is 200 mg/kgbw.

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