# Activity Test of CLA Syntheszed from Castor Oil by in Vivo White Mice (*Rattus norveginus*)

# Activity Test of CLA Syntheszed from Castor Oil by in Vivo White Mice (*Rattus norveginus*)

**Marham Sitorus[1],*, Manihar Situmorang[1], Tita Juwitaningsih[1], Minat Karya Nia Hia[1]and Rini F Hutabarat[1]**

[1]Department of Chemistry,Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Medan 20221, Indonesia

*Corresponding author: marham@unimed.ac.id

**Abstract.** Malondialdehyde (MDA) is a metabolite resulting from lipid peroxidation by free radicals that can be formed when hydroxyl free radicals such as Reactive Oxygen Species (ROS) react with fatty acid components of the cell membrane so that a chain reaction is known as fat peroxidation. The fat peroxidation will break the chain of fatty acids into various toxic compounds and cause damage to the cell membrane. Thus MDA is an parameter of the presence of free radicals in the body. In this research, the Conjugated Linoleic Acid (CLA) antioxidant test was synthesized from castor oil in vivo against white mice (*Rattus norveginus*). White mice exposed to free radicals through cigarette smoke for 2 hours per day for 14 days. Mice were given an antioxidant intake of CLA with a concentration of 200, 400, 600,800,1000 mg / body weight every day with three repetitions. The same thing is done with intake of Ascorbic acid (Vitamin C) and α-tocopherol (Vitamin E) as a comparison. As a control also made negative control without treatment and positive control by exposure to cigarette smoke without intake of antioxidants. The CLA can inhibit free radicals by reduction MDA in blood an inhibitory equivalent to Ascorbic acid (Vitamin C) and α- tocopherol (Vitamin E).

## 1. Introduction

Antioxidants are compounds that can inhibit the rate of oxidation or prevent the reaction of free radical formation by neutralizing free radicals in cells. Chemically free radicals cause cell damage and will cause cancer and tumors, which today is one type of disease that causes high mortality [1]. Compounds that are widely used as antioxidants are α-tocopherol or vitamin E and ascorbic acid or vitamin C, which are abundant in nature, whereas when synthesized requires high costs.

The urgency of this research is the study of alternative antioxidants that are effective, inexpensive, abundant and renewable. Conjugated Linoleic Acid (CLA) which is found in large livestock (ruminant), milk and its by-products are also antioxidants, but the abundance is very limited. Synthetically, CLA can be obtained from ricinoleate, which is a major component of castor oil. Castor oil is obtained from castor bean seeds (*Ricinus communis* Linn) with oil content of 40-50% and ricinoleic content of 80-90%, castor oil is one of the oil that is not consumed (non edible oil) and this is an advantage because it does not compete with edible oil [2]. In vitro the antioxidant activity of Conjugated Linoleic Acid (CLA) synthesized from castor oil has been tested against 4 µg / mL α, α-diphenyl-2-picrikhidrazyl (DPPH) with ethanol as a spectrophotometer[3]. The results show that at

concentrations starting at 4 µg / mL, it can inhibit more than 80% DPPH equivalent to the inhibitory power of α-tocopherol and ascorbic acid which is a commonly used natural antioxidant. The IC50 (inhibitory concentration of 50%) of CLA measured at 8 µg / mL DPPH is 2,133 4 µg / mL equivalent to α-tocopherol 2,839 4 µg / mL and ascorbic acid 2,947 4 µg / mL. The very high antioxidant activity of CLA and the very strong IC50 category (<50 µg / mL) against DPPH indicate that CLA has the potential to be used as an antidote [3].

Furthermore, in vivo, the antioxidant activity of CLA has been tested on experimental animals which are conditioned to be exposed to free radicals through cigarette smoke by measuring blood Malondyaldehide (MDA) levels, spectrometers appear as parameters that animals are exposed to free radicals. The presence of MDA in the blood is the result of free radical lipid peroxidation as an indicator of the presence of free radicals in the body of experimental animals [4, 5, 6, 7].

## 2. Materials andMethods

### *2.1. Chemicals and instruments*

Chemicals materials used are qualification Brandsnamely:Tiobarbituricacid (TBA), Trichloroacetic (TBA), Acetic acid glacial, 11,33, Tetra methhoxy propane (TMP), Aquadest and white mice (*Rattus norvegicus*). The research instrument are, EDTA tube (vortex), smoking chamber, centrifuge and UV – Vis SIMADZU Spectrophotometer.

### *2.2. Activity test of CLA syntheszed from castor oil by in vivo white mice (Rattus norveginus)*

The experimental animals (*Rattus norvergicus*) used were 2.5 - 3.0 months old, body weight 100 - 200 g, active, not deformed and male. Animal experiments were divided into 7 treatment groups randomly. Each treatment group consisted of 3 replications for 3 types of antioxidants (CLA, Vitamin C and D), so that the total number of mice used was 65 animals including positive and negative controls. The cigarettes used are clove cigarettes. Free radical exposure was carried out using 3 cigarettes for each treatment group, except the negative control group. The exposure was carried out for 14 days in a smoking chamber. One hour after exposure to cigarette smoke continued with given of CLA, Vitamin C and Vitamin D according to the dose in each treatment group. Furthermore, blood sampling was performed on rats that were fasted for 24 hours. Mice were anesthetized using chloroform then dissected and their blood drawn through the heart organ using a 3 mL syringe. The blood obtained is collected in a blood tube and centrifuged for 10 minutes at 3000 rpm. Blood plasma located at the top is separated and taken to be analyzed for MDA concentration with Vissible spectrophotometer at wavelength ($\lambda$ = 535 nm).

## 3. Results and Discussion

The initial stage carried out in this in vivo test was the conditioning of mice in the experimental cage before treatment. After conditioning the exposure of cigarette smoke with a duration of 2 hours / day for 14 days and continued with the treatment of intake of CLA, as cornic acid (vitamin C) and α-tocopherol (vitamin E) as a comparison and ascorbic acid with a variation of concentration of 200, 400, 600, 800 and 10 mg / kg / day with three repetitions, respectively. Besides that, treatment was also carried out for positive control (fumigation without antioxidant intake) and negative control (without fumigation and without antioxidants). After completion of the treatment, blood from the heart of the experimental animal is then surgically collected in an EDTA tube to prevent clotting and then centrifuged for blood serum collection [8, 9].

**Table 1.**Absorbance data and antioxidant inhibition of CLA, ascorbic acid (vitamin C) and α-tocopherol (vitamin E).

| Dosage (mg/kgWeight) | CLA | | Asam Ascotbat (Vit C) | | A-tokoferol (Vit D) | |
|---|---|---|---|---|---|---|
| | $A_{av}$ | %inhibition*) | $A_{av}$ | % inhibition*) | $A_{av}$ | % inhibition*) |
| 200 | 0,0493 | 3,52 | 0,0413 | 19,18 | 0,0477 | 6,65 |
| 400 | 0,0482 | 5,68 | 0,0468 | 8,41 | 0,0474 | 6,85 |
| 600 | 0,0474 | 7,63 | 0,0481 | 5,87 | 0,0477 | 6,65 |
| 800 | 0,0478 | 6,46 | 0,0483 | 5,48 | 0,0479 | 6,26 |
| 100 | 0,0447 | 12,52 | 0,0465 | 9,01 | 0,0446 | 12,72 |

$A_{controle(-)} = 0,0397$
$A_{controle(+)} - A_{controle(-)} = 0,0114$ *(MDA in the body)*
**) % inhibition* $= [(A_{controle(+)}) - (A_{antooksidant}]/(A_{kontrol\ (+)}$

Furthermore, measured levels of Malondyaldehide (MDA) blood serum, by spectrometer appear. Malondialdehyde (MDA) is a metabolite resulting from lipid peroxidation by free radicals that can be formed when hydroxyl free radicals such as Reactive Oxygen Species (ROS) react with fatty acid components of the cell membrane so that a chain reaction is known as fat peroxidation. The fat peroxidation will break the chain of fatty acids into various free radicals that are toxic and cause damage to the cell membrane. Thus MDA is an indicator of the presence of free radicals in the body with the results in Table 1 and the graph in Figure 1.

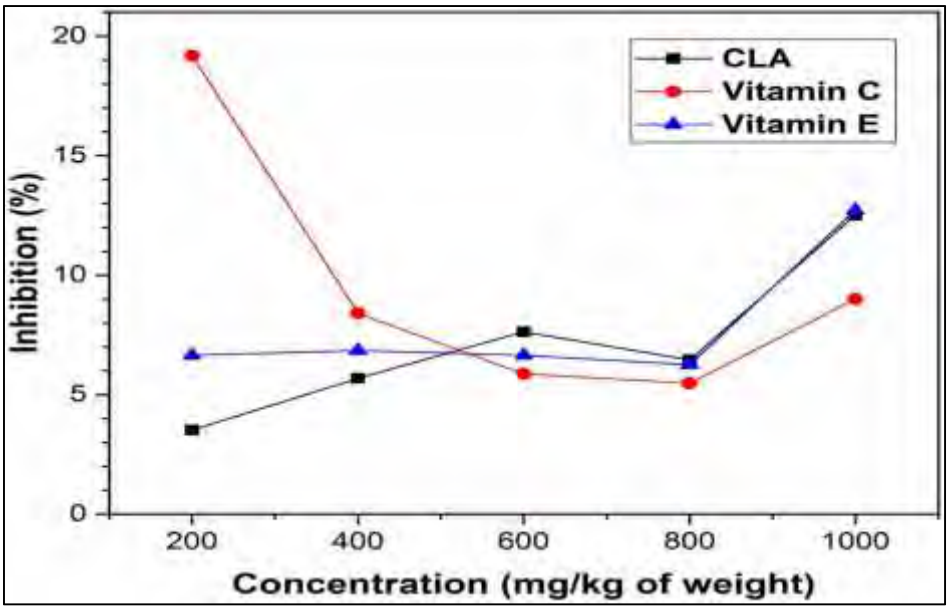


**Figure 1.** Graph of the inhibitory power of antioxidant CLA, ascorbic acid (vitamin C) and α-tocopherol (vitamin D) at concentration (mg / kg weight).

Based on table 1 and figure 1, the CLA can inhibit free radicals and the relative pattern is the same as the inhibitory power of ascorbic acid (Vitamin C) and α-tocopherol (Vitamin E).Thus the CLA has the potential to be used as an inexpensive and abundant natural antioxidant. In addition it is also a renewable antioxidant, through the cultivation of Ricinus tree as a source of castor oil. Many plants are easy to grow because they do not need strict soil requirements and can grow on marginal land. The contrribution will raise the economic value of the castor plant in general and castor oil in particular.

## 4. Conclusions

As in vitro CLA can inhibit DPPH with $IC_{50}$ (inhibitory concentration of 50%) 2,133 4 µg / mL equivalent to α-tocopherol 2,839 4 µg / mL and ascorbic acid 2,947 4 µg / mL. The very high antioxidant activity of CLA and the very strong $IC_{50}$ category (<50 µg / mL) against DPPH indicate that CLA has the potential to be used as an antioxidant. Furthermore, the antioxidant activity in vivo to experimental animals conditioned by free radicals can inhibit free radicals measured by decreasing blood Malondyaldehide (MDA) by vissible spectrometers as parameters that animals are exposed to free radicals.

## References

[1] Yu L, Adams D and Gabel M. 2002 Conjugated linoleic acid isomers differ in their free radical scavenging properties *Journal of Agricultural and Food Chemistry***50** 4135-4140.
[2] Patrick L. 2006 Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity *Alternative Medicine Review***11** 114-127.
[3] Bhowimic D N and Sarma A N. 1987 Dehydration of castor oil**.**Ind. Eng. Chem. Prod; Departement of Oil and Paint Technology Kampur India.
[4] Muniz P, Coma M J and Teran J. 2014 Oxidative stress and vascular damage in hypoxia processes. Malondialdehyde (MDA) as biomarker for oxidative damage *Electron J Biomed***2** 50-53.
[5] Sinaga F A. 2017 Stress oksidatif dan status antioksidan pada aktivitas fisik maksimal *Generasi Kampus* **9** 176-189
[6] Sas K, Robotka H, Toldi J and Vécsei L. 2007 Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders *Journal of the Neurological Sciences***257** 221-239.
[7] Raharja K T Wirjatmadi B and Adriani M. 2016 Pemberian buah kawista menghambat peningkatan kadar malondialdehid serum tikus wistar yang dipapar asap rokok *Jurnal Kedokteran Brawijaya,* **29** 196-201.
[8] Pryor WA. 1997 Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity *Environmental health perspectives***105** 875-882.
[9] Tyndale R F and Sellers E M. 2001 Variable CYP2A6-mediated nicotine metabolism alters smoking behavior and risk *Drug Metabolism and Disposition***29** 548-552.
[10] Simorangkir M, Hutabarat W, Nainggolan B and Silaban S. 2019 Antioxidant and antibacterial activities of nonpolar to polar solvent extracts of Sarang Banua (*Clerodenrumfragrans* Vent Willd) leaves *Rasayan Journal of Chemistry***12** 959-965.

**Acknowledgement**
Research grant from Directorat General of the Ministry of Research, Technology and Higher Education who funded this research through the Grant of Centralization of Competency-Based Finantial Year 2019 is gretefully acknowledged. Thanks also to Dr.Tutik Dwi Wahyuningsih, M.Sc Head of the Organic Chemistry Laboratory and Instrument Gajah Mada University who recorded, GC-MS and FTIR and Drs. Marudut Sinaga, M.Si, Head of the Unimed Chemistry Laboratory which records the antioxidant activityby UV Spectrophotometer. Thank you also disguised to Minat Karya Nia Hiaand Rini F Hutabarat students who were involved in this research in order to completedher Thesis.