Effect of Centella Asiatica on Oxidative Stress in Rat Lung after Formalin Exposure

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***Abstract*—Pegaga or *Centella asiatica* commonly used in traditional medicine was believed to possess antioxidant effect that can control the generation of free radicals. This study aims to investigate the protective effect of *Centella asiatica* on antioxidant status in rat lung following formalin exposure. Twenty male Wistar rats were divided into four groups: (1): control; (2): exposed with 10 % formalin; (3): exposed with 10 % formalin and treated with ethanolic extract of *C. asiatica*; (4): treated with ethanolic extract of *C. asiatica*. Exposure of 10 % formalin was performed through inhalation and *C. asiatica* was given orally. After the treatment, the rat's lungs were harvested for determination of malondialdehye and activities of superoxide dismutase and catalase. Exposure to 10 % formalin did not increase the concentration of malondialdehye. However, higher malondialdehye level was noted in group which exposed with 10 % formalin and received ethanolic extract of *C. asiatica*. A significant decrease of superoxide dismutase activities was observed in rat's lung between all groups as compared to control group, yet no significant difference was observed in catalase activities. In conclusion, exposure 10 % formalin was unable to induced oxidative stress in rat lung but supplementation of *C. asiatica* are able to increase superoxide dismutase level in rat lung but not for catalase.**

***Index Terms*—centella asiatica, formalin, lung, oxidative stress, antioxidant.**

I. Introduction

The *Centella asiatica* (*C. asiatica*) belongs to the family of Apiaceae or Umbelliferae, has been used to treat a wide range of illness [1]. This medicinal plant is widely spread in many Asian countries and consumed by local people freshly. The plant extract has been incorporated into the Indian pharmacopoeia and recommended not only for wound healing but also for the treatment of skin diseases such as eczema, leprosy and psoriasis [2].

Natural antioxidants, especially from plant origin, has notably increased in recent years but so far few attempts has evaluated therapeutic intervention with the natural antioxidant like *C. asiatica*. Different parts of *C. asiatica* contain different amount of phytochemicals. Phytochemicals in *C. asiatica* have been recognized to posses many properties including antioxidant, anti-allergic, anti-inflammatory, anti-viral, anti-proliferative and anti-carcinogenic effects [3]. Among the root, leaves and petiole of *C. asiatica*, the leaves exhibits higher antioxidant activity compared to other parts [4].

Antioxidants are chemicals that interact with and neutralize oxidant, free radicals or reactive oxygen species (ROS) produced in the body. These free radicals have the potential to harm cells. Increase production of free radical, leads to an imbalance steady state in the body known as oxidative stress. Oxidative stress occurs due to imbalance between production and detoxification of ROS. Increased oxidative stress will induce lipid peroxidation, DNA damage, protein damage and induction of apoptosis which will result in cell death [5]. Naturally, minimal amount of oxidants are produced in our body resulted from cell's metabolism. This minimal amount of oxidant did not affect our body significantly as it will be cleared out efficiently. However, the amount of oxidant will accumulate, increasing and affect the body system once the body is exposed to some causative agent of oxidative stress in high concentration or prolonged exposure.

Formaldehyde (FA) derived from chemical formula CH_2O with molecular weight of 30.03 in a form of powder. FA appear colorless when evaporate. It is broadly used in hospitals, laboratories and industrial settings [6]. It also has been used in daily life such as in perfume, paint and shampoo and in many other products such as disinfectants, cosmetics, antiseptics and fungicides [7]. FA is commercially available as a solution called formalin, formed from various proportions of formaldehyde, water, and alcohol as stated in Occupational Safety and Health Administration [8].

Based on the available evidence in Occupational Safety and Health Administration [8] record on formaldehyde, OSHA has determined that formaldehyde is genotoxic, showing properties of both a cancer initiator and promoter (early and late stage carcinogen). When inhaled, formaldehyde is a carcinogen in rats. In humans, formaldehyde exposure has been associated with cancers of the lung, nasopharynx and oropharynx, and nasal passages. When humans are exposed to excess levels of formaldehyde, many adverse health effects can be observed. Symptoms of excess exposure includes respiratory irritation (watery), itchy eyes (itchy, runny, or stuffy nose), dry or sore throat and headache.

Single exposure with lower dose may not show a significant effect. However, longer exposure to FA with

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minimal dose may develop oxidative stress and subsequently lead to serious organ injury. According to Hazard Evaluation System & Information Service (HESIS) in year 2011 [9], exposure to high levels of FA (5-30 ppm and higher) can severely irritate the lungs, causing chest pain and shortness of breath. Repeated exposure to FA can cause allergic asthma in many cases. Symptoms of asthma include chest tightness, shortness of breath, wheezing, and coughing.

This paper is aim to investigate the protective effects of *C. asiatica* toward formalin-induced oxidative stress in rat lung by determines the level of malondealdehyde activities and antioxidant enzyme (superoxide dismutase and catalase).

II. Methodology

A. Sample Preparation

Two kilograms of selected *C. asiatica* were obtained from fresh local produce. About 250 g of *C. asiatica* leaves were grounded and extracted by ethanol for 48 hours. The solvent was removed by using rotary evaporator (40 ℃). The final product of extraction is a pure *C. asiatica* appears in dark green color.

B. Preparation of Formalin

A commercially available 37 % formaldehyde solution (R & M Marketing, Essex, UK) was further diluted in isotonic saline. In this study, 4 % formaldehyde or 10 % buffered formalin is commonly prepared by adding 100 ml of 40 % formaldehyde to 900 ml distilled water with 4 g sodium phosphatase, monobasic and 6.5 g sodium phosphate, dibasic (anhydrous).

C. Animal Preparation

We obtained approval from our Institution's Research and Ethics Committee. The usage of animals for this project has been approved by the Institution's Animal Ethics Committee. A total of 20 healthy adult male Wistar rats (150-200 g) were used in this study. The animals were housed in plastic cages (2 rats per cage). The animals were maintained under controlled temperature (21-23 ˚C) and 12 h light/12 h dark cycle conventional conditions, with free access to food and water. All animals were received human care according to the criteria outline in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

D. Exposure to Formalin

Animals used were divided into two groups which are: 1) expose to FA (n=10) and 2) do not expose to FA (n=10). The groups which exposed to 10 % formalin were subjected to daily 10 % of FA inhalation for 4 hours/day, 5 days/week for subsequently 8 weeks, with each of the group was given the same volume of formalin. The formalin test was carried out in clear transparent plastic boxes to generate a constant airstream from an aqueous solution of formalin. The weight and physical observation of rats was recorded in daily basis.

E. Supplementation of C. asiatica

Upon exposure period, rats were further subdivided into two groups and final groups were as follows: (1): control (received drinking water); (2): exposed with 10 % formalin (4 hrs/day, 5 days/wk, 8 wks); (3): exposed with 10 % formalin (4 hrs/day, 5 days/wk, 8 wks) and treated with 50 mg/kg b.w of ethanolic extract of *C. asiatica* (14 days); (4): treated with 50 mg/kg body weight of ethanolic extract of *C. asiatica* (14 days). Ethanolic extract of *C. asiatica* was given orally.

F. Biochemical Analysis

Upon exposure and treatment period, all rats were sacrificed and the lungs were harvested. Lungs of each rat were weighted and divided equally for biochemical analysis. Then, each small pieces of lung was immediately frozen in liquid nitrogen to stop the biochemical reaction in organ. Each of lung's portions was analyzed for malondialdehyde (MDA) assay [10], superoxide dismutase (SOD) assay [11], catalase (CAT) assay [12] and protein estimation [13].

G. Statistical Analysis

All statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) statistical software version 17.0. Data was expressed in mean ± standard error of the mean (S.E.M). Two-way ANOVA was used to compare means among four experimental groups. Post-Hoc Dunnet test was used to make the comparison between means. p value less than 0.05 will considered as significant.

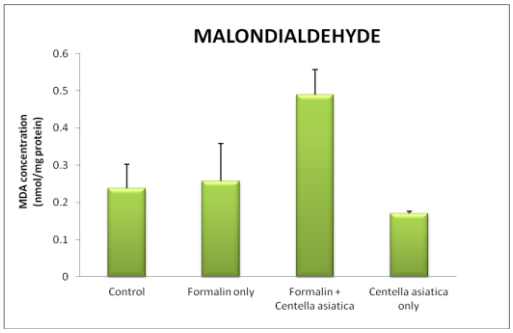

Figure 1. Level of MDA activities in all experimental groups. There is significant different ($p<0.05$) between expose to 10 % of formalin and received ethanolic extract of *C. asiatica* treatment group and group which given ethanolic extract of *C. asiatica* treatment only.

III. Result

A. Malondialdehyde Concentration

Fig. 1 shows the malondialdehyde concentration in all experimental groups. From this study, there is no significant differences ($p>0.05$) for the MDA concentration level of rat's lung between all group compared to control group. When comparing each group to another, significant increase of MDA concentration was seen in group between exposure with 10 % formalin and received ethanolic extract of *C. asiatica* treatment group (0.48880 ± 0.06792 nmol/mg protein) as compared to group that administrated with *C. asiatica* only

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(0.16960 ± 0.00649 nmol/mg protein). However, there was an increasing pattern in MDA concentration in group which were exposed to 10 % formalin, but it is not significant.

B. Superoxide Dismutase Activity.

Fig. 2 shows the superoxide dismutase activity in all experimental groups. From the graph, the activity of superoxide dismutase for the rat's lung in the group that exposed to the 10 % formalin group (1.9576 ± 0.394623 U/mg protein), group that has been exposed to 10 % formalin and received ethanolic extract of *C. asiatica* (2.548 ± 0.350132 U/mg protein) and group that only received ethanolic extract of *C. asiatica* (3.15320 ± 0.295732 U/mg protein) was significantly decreased ($p<0.05$) when compared to control group (4.89620 ± 0.332812 U/mg protein).

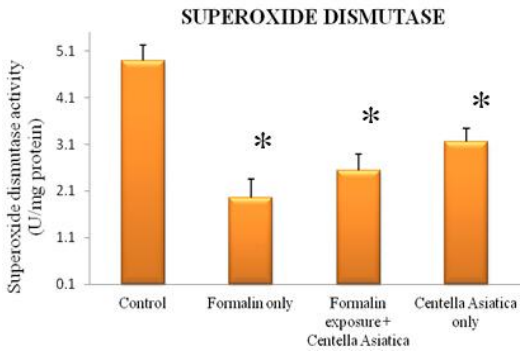

Figure 2. Level of SOD activities in all experimental groups. There is significant different ($p<0.05$) between group that exposed to 10 % formalin, group that exposed to 10 % formalin and administrated with *C. asiatica* and group that only administrated with ethanolic extract of *C. asiatica* as compared to control group.

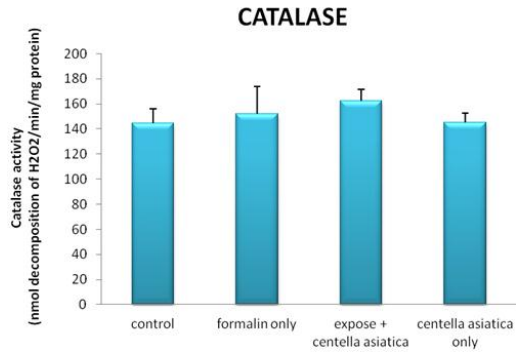

Figure 3. Level of CAT activities in all experimental groups.

C. Catalase Activity.

Fig. 3 shows the catalase activity in all experimental groups. From this study, there was no significant differences ($p>0.05$) was seen in all groups as compared to control. However, catalase activity was seen higher in group that exposed with 10 % formalin and recieved *C. asiatica*.

IV. Discussion

This present study was conducted to determine the antioxidant activities of *C. asiatica* towards formalin-induced oxidative stress in rat's lung. The biochemical analysis showed that the exposure to 10 % formalin for 4 hours/day, 5 days/week for 8 weeks did not significantly increase the level of MDA in rat's lung. This result is in contrast to a study done by Sul *et al.* in year 2007 [14]. Their finding showed that when the rats were exposed with two different concentrations of formaldehyde (0, 5, 10 ppm) for 2 weeks at 6 h/day and 5 days/week in an inhalation chamber, there was a significant increase of MDA level, carbonyl insertion and DNA damage in the lungs of rats. Even though there is no significant different in MDA concentration level between control and formalin only group, the result showed an increasing pattern of MDA concentration level. This may due to small sample size and increasing sample size might results in significant changes. On the other hand, the used of 10 % formalin in inducing oxidative stress was adopted from a study by Golalipour *et al.*, (2007) [15]. The rats was left in the cadaver's room for 18 weeks and resulted in significant increase of MDA concentration in rat testis relative to the control group. However, the same observation was not detected in the present study. This contrast may be due to differences in studies organ and the duration of exposure, as in this study the duration of exposure was shorter than those reported by Golalipour *et al*., (2007) [15].

Previous study explains the lung's defense mechanism as the lung itself contain intracellular antioxidant enzyme to maintain a normal redox state. The alveolar space can recruit additional antioxidant activity from the epithelial lining fluid [16]. This fluid contains large amounts of GSH CAT, SOD, and GPx [17]-[20] making lung's defense mechanism very efficient. This might be the possible explanation why the level of MDA was not significantly increase in group of rats exposed to 10 % formalin.

Higher level of MDA concentration was noted in group exposed to 10 % formalin and supplemented with ethanolic extract of *C. asiatica*. This finding reveals the adverse effect of supplementation of *C. asiatica* upon formalin exposure. Observation by Shelley Moore (2010) [21] from University of Maryland Medical Center (UMMC) showed that a main component of *C. asiatica* called asiaticoside has been associated with tumor growth in mice. However, limited study was done to prove this statement. Further research needs to be done to investigate the effect of asiaticoside in enhancing tumor growth and most probably as medication of higher oxidative damage.

In this study, exposure to 10 % formalin resulted in significant decrease of SOD activity in rat's lung. This result is supported by finding from Lino-Dos-Santos-Franco *et al.* (2010) [22] which concluded that formaldehyde cause a decrease in SOD activity when compared to the naïve group. SOD is a ubiquitous enzyme with an essential function in protecting aerobic cells against oxidative stress by catalyzing superoxide (O_2^-) radicals to hydrogen peroxide (H_2O_2) [23]. Therefore, SOD converts two superoxides into hydrogen peroxide and oxygen making it is less toxic than

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superoxide. When there is oxidative stress resulted from formaldehyde exposure, this enzyme directly catalyzed the formation of free radicals hence reduced the level of superoxide dismutase.

Higher level of SOD activity was noted in control group as compared to other group. This is because; SOD is a primary defense mechanism of human lung against free radical. SOD was commonly found higher in rat's lung [24]. Moreover, high level in SOD activity was seen in group which received with *C. asiatica* only. This proved that the ethanolic extract of *C. asiatica* possess an antioxidant property which supported by study done by Hashim (2011) [25]. Higher activity of SOD was also seen in group exposed to 10 % formalin and supplemented with ethanolic extract of *C. asiatica* when compared to group exposed to 10 % formalin only. This showed that exogenous SOD from *C. asiatica* is able to enhanced endogenous SOD activities in reducing oxidative damage induce by formaldehyde.

Catalase was crucial to detoxify hydrogen peroxide that is formed by the reaction of superoxide dismutase enzyme toward superoxide anion and has no electron donor requirement. Catalase is a well-known antioxidant enzyme and has proven to protect the body from hydrogen peroxide. Superoxide dismutase and catalase will form subsequently in body defense system to fight with the formation of oxidative stress [26]. Naturally, lung contains an efficient defense mechanism other than enzymatic antioxidant. This is due to its normal physiology of the lung itself. It contain intracellular antioxidant enzyme which can maintain a normal redox state. The alveolar space can recruit additional antioxidant activity from the epithelial lining fluid [16]. In relation to this study, differences in catalase activity were not significantly detected in all experimental groups. This is may be due to role of converting hydrogen peroxide has been taken up by GPx as this enzyme are abundant in lung and also catalyzing hydrogen peroxide to oxygen and water [17]-[20].

V. Conclusion

Exposure to 10% Formalin was unable to induced oxidative stress in rat lung. This is due to characteristic of lung itself as a primary defense mechanism in human but the supplementation of *Centella Asiatica* is able to increase SOD level in rat lung. However, not for catalase.

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