

## Formalin Exposure on the Rats Feeding Diet on Antioxidant Enzymatic activity and Oxidative Damage of Rats Liver Tissue

Chanif Mahdi and Aulanium

Biochemistry Laboratory of Chemistry Department Faculty of mathematic and Natural Science University of Brawijaya Malang

HP: +628125297102

Email: [chanifmahdi@gmail.com](mailto:chanifmahdi@gmail.com); [chanif@ub.ac.id](mailto:chanif@ub.ac.id)

**Abstract.** Using formalin or formaldehyde as a illegal preservative on food and ingredient Is very danger for health because formalin is a toxic and carcinogenic substance that potent as a sources of reactive oxygen species (ROS) and free radical exogenous. The aim of this research was to know the potent of formalin toxicities that exposure through the rats feeding diet on antioxidant enzymatic activity (SOD and GSH) and effect on oxidative damages of rats hepar tissue (MDA). Twenty five of male rats of 8 to 10 weeks old, with the body weigh 100 to 120 g, were divided into 5 groups. Group I was the control group, treated with a standard pellet feeding diet without formalin. Group II, III, IV and V were administrated with treatment feeding diet with formalin content of each were 25 ppm, 50 ppm, 75 ppm and 100 ppm. The result showed that formalin exposure through the feeding diet of rats (*Rattus novegicus*) affect decreasing highly significantly ( $P < 0,01$ ) on antioxidant enzymatic (GSH) and increasing oxidative damage (MDA) of liver tissue of rat (*Rattus norvegicus*).

**Keywords:** Formalin; Superoxide dismutase (SOD); Glutathion reduced (GSH); Malonyl dialdehyde (MDA).

### Introduction

Formalin or formaldehyde as an illegal food preservative is very danger for body health, because formalin is a toxic and carcinogenic substance and as sources of Reactive Oxygen Species and free radical substances. In 198 The U.S. Environmental Protection Agency (EPA) classification formaldehyde or formalin as probable human carcinogen under condition unusually high or prolong exposure. Since that time, some studies oh humans have suggested that formaldehyde exposure is associated with certain of cancer. The International Agency for Research on Cancer (IARC) classifies formaldehyde as a human carcinogen.

In Indonesia as already known that, formalin or formaldehyde has been widely used as a illegal foods preservative. For example Formaldehyde is widely used as illegal preservative in i wet noodle industry, dried and fresh fish industry, meat ball industry, tofu industry an soon. So to day The Indonesian community seems difficulty to obtain food and foods ingredient are fully

free of illegal preservative substance (Formalin).

Formalin or formaldehyde is a simplest organic compound of aldehyde or alkanal group. Formaldehyde is a toxic and carcinogenic substance. Formaldehyde contamination through the food or feeding with high doses level is very danger for the body health. Formaldehyde is a reactive compound, its easy to react to nucleofilic groups, especially to  $\text{NH}_2$  group of protein (enzyme system). That can affect on decreasing specific activity of enzyme, especially of oxidative phosphorylation system of cytochromP<sub>450</sub> disturbs. Overall of this reaction will affect to acidosis condition of the cells, tissue and body organs, and tendency to over production of reactive oxygen species (ROS) and free radical substances, as a result of disturbed of Beta-oxidation cycle, and it will affect to decrease ATP production and tendency to dead cells necrosis (Lee, 2003 and Murray, 2005).

Over production of reactive oxygen species (ROS) and free radical substances will affect of both to cell membrane damages and

mitochondria and canal ion damages. Ions  $\text{Ca}^{2+}$  accumulated in cytosol, and stimulated NF- $\kappa$ B were active. This condition stimulate inflammation of the cells and tissue of organs, that characterized generation of radical nitrooxide (NO.), and it will reaction to radical superoxide ( $\text{O}_2^-$ ) to form peroxinitrit radical that have potent more toxic (Bray, 2006; Dassagayam et.al; Lee, 2003).

Fifty degenerative disease affect by ROS and free radical for instance are: Hepatitis, cataract genesis, Age pigment, Heart attack, Arthritis, Asthma, stroke, cancer, Atherosclerosis, periodentis, and soon (Kumar, et al 2003; and Teng, et al 2001).

## Material and Method

### Chemicalian

Chemicalian: Formaldehyde, yoghurt, tyrosine, hematoxylen, eosine, etanol 98 %, SDS, acrylamid, bis acrylamid, glycerol, aquadest, xillol, parafine, bromophenol blue, commasive, brilliant blue.R. 350, tris bas, B-marcaptoetanol, amonium persulfat, NaCl, KCl,  $\text{Na}_2\text{PO}_4$ ,  $\text{KH}_2\text{PO}_4$ , TCA, TBA, HCl, aquabidest, MDA Kit. All of chemicalian are pro Analysis qualification (Merck Production)

### Instruments

Analytic Balance AE 50 ; Spectrophotometer UV-Vis ; electrophoresis; Vortex Gua Hug; Spoid 1 mL; Gavages; Volumetric Glasses; Water Bath, Micro pipette; Micro Tip; Eppendof; Rotary microtome.

### Animal Experiment

Twenty five of 8 – 10 weeks old male rats (*Rattus norvegicus*), with the body weigh 100 – 120 g, were divided equally into 5 groups, each of group contain 5 rats. Group A was t control; Group B, C, D and E were administrated with formalin treatment of each were 25 ppm; 50 ppm; 75 ppm and 100 ppm. All of each group without and supplementation before and after formalin treatment.

## Method

### Histopathology observation

Hepar tissue were fixed with 4 % of buffer formalin two times for 24 hours. Hydrated tissue using alcohol rise, and then inserted into xyllen twice. Incorporated gastrointestinal tissue into soft paraffin, then into hard paraffin, then on the block paraffin and allowed to stand at room temperature. Cutting the tissue is done by using a rotary microtome with thickness of 10  $\mu\text{m}$ . Demounting on the glass slide using Mayer albumin. The preparation is then stained with Eosin hematoxylen. Staining and preparation were incubated into Mayer hematoxylen. Further preparation were washed with water and be dehydrated with alcohol rise. The preparation was washed with water and dried amounted with entelen ( Biomedical Laboratory, 2004)

### Measurement superoxide dismutase (SOD) Activity

A total of 100mg of rat hepar tissue was put in a mortar, crushed into powder form and

added with one mL of phosphate buffer saline pH 7.4. Homogenate formed put in propylene tubes and centrifuged at 4000 rpm at 4°C in 15 minutes. 500 $\mu\text{L}$  of supernatant added 25mM EDTA, NTB 25 unit/mL, 25mM Xanthine oxidase 1 unit/mL, 100 $\mu\text{L}$  respectively. Then the mixture solution added with PBS pH 7.4 and homogenized with vortex. After it was incubated at 35°C in 30 minutes and cooled at room temperature. Then centrifuged at 3500 rpm for 10 minutes at room temperature. Furthermore, the supernatant absorbance was measured with a spectrophotometer at the maximum wavelength (580 nm). The results are plotted on a standard curve that has been made to calculate the activity of SOD [ Laboratorium Farmakologi 2007).

### Measurement of Glutathion (GSH)

Weighed as much as 400 mg Rats hepar tissue, Coupled with 2 mL of sulfonic acid, then homogenized. The homogenate centrifuged at 600 rpm for 10 minute at 4°C . The supernatant taken many as 250  $\mu\text{L}$ , reacted with DTNB. The solution absorbance was measured using a spectrophotometer at the maximum wavelength (415 nm). The

result are plotted with standard curve, to calculate the levels of GSH [ Biooxytech, 2008)].

### Measurement of Malondialdehyde (MDA)

As much as 1.8 g of rats hepar tissue was cut into small piece, then crushed in mortar, placed on cold block of ice. Then add 1 mL of NaCl 9.0 percent. Homogenate transferred into micro tubes and centrifuged at a speed of 8000 rpm for 20 minutes. 100  $\mu$ L of supernatant was added 550  $\mu$ L distilled water. Then 100  $\mu$ L of Na- thiobarbituric acid. At each reagent homogenized with vortex. Then centrifuged at 500 rpm for 10 minutes. Supernatant was taken, transferred to new tubes. The solution was further incubates in the water bath with temperature 100<sup>o</sup> c FOR 30 minutes, at left at room temperature.

Furthermore, the supernatant absorbance was measured with a spectrophotometer at maximum wave length (530 nm), and plotted on standard curve that has been made to calculate the concentration of MDA (Farmakology Laboratory, 2007).

## Result and Discussion

### 1. Effect formaldehyde exposure on antioxidant activities of hepar rats tissue

Table 1. Effect of formaldehyde exposure through the feeding diet of rats on SOD activities.

Formaldehyd treatment	SOD Activities (Unit)						Mean Value	SOD activities (%)
Kontrol (0 ppm)	70,66	73,05	70,47	72,55	70,93	71,53 <sup>e</sup>	100	
25 ppm	28,59	29,27	29,70	29,23	28,87	29,13 <sup>d</sup>	40.72	
50 ppm	24,56	26,25	27,39	26,57	25,25	26,00 <sup>c</sup>	36.35	
75 ppm	20,15	21,05	21,05	19,78	19,89	20,38 <sup>b</sup>	28.49	
100 ppm	17,21	16,89	17,11	16,91	17,17	17,06 <sup>a</sup>	23.81	

**Table 2** Effect of formalin exposure through the feeding diet on GSH activities

Formaldehyd treatment	GSH ( $\mu$ g / ml )					average	GSH actives (%)
Kontrol (0 ppm)	50,23	50,41	50,11	51,13	50,43	50,26 <sup>e</sup>	100
25 ppm	25,07	25,21	25,21	25,33	25,41	25,25 <sup>d</sup>	50.39
50 ppm	21,11	21,25	21,13	21,27	21,33	21,20 <sup>c</sup>	42.18
75 ppm	17,27	17,33	17,19	17,17	17,29	17,23 <sup>b</sup>	34.40
100 ppm	13,67	13,71	13,63	13,57	14,07	13,73 <sup>a</sup>	27.32

Datas from Tables 1. And Table 2 show, that increasing doses level of formaldehyde treatment lead to decrease of SOD and GSH activity and body immunity of rats. This happening, because Formalin or formaldehyde is a toxic and carcinogenic substance as a sources of ROS and free radical compound that can affect to decreasing antioxidant enzymatic for instance of both SOD and GSH activities. Its according suggestion by Lee (2003) and Murray 2005) that , Formaldehyde or formalin is a simplest organic compound of aldehyde or alkana group is a toxic and carcinogenic substance. Formaldehyde contamination through the food and feeding diet with high doses level is very danger for the the body. Formaldehyde presenting in the body will affected decreasing of reactive Oxygen species (ROS), and series reaction to product new ROS and free radicals substances that more reactive and more toxic and more danger to affect damage and dead cells.

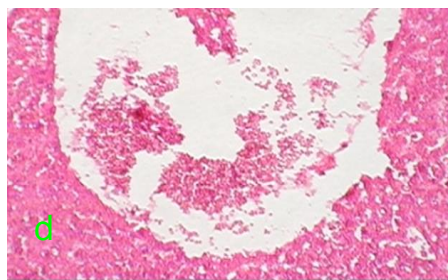
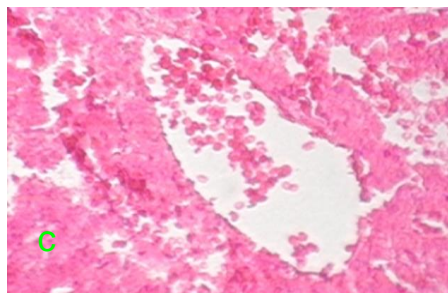
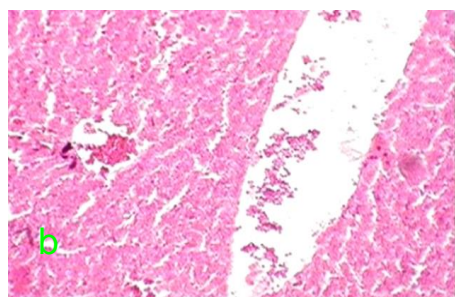
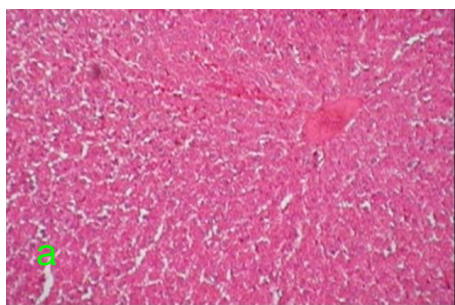
Formaldehyde is a reactive compound , its easy to react to nucleophilic groups , particularly to NH<sub>2</sub> group of protein or enzyme system , that can affect on decreasing specific activity of enzyme, particularly of oxidative phosphorylation system of cytochrome P450 disturbed, Including SOD and GSH as a antioxidant enzymatic. Its according suggestion of Lee (2003) and Bray (2005), Formalin or formaldehyde is a simplest organic compound of aldehyde or alkanal group. Formaldehyde is a toxic and carcinogenic substance. Formaldehyde contamination

through the food or feeding with high doses level is very danger for the body health. Formaldehyde is a reactive compound, its easy to react to nucleofilic groups, especially to NH<sub>2</sub> group of protein (enzyme system). That can affect on decreasing specific activity of enzyme,

## 2. Effect of formaldehyde exposure through the rats feeding diet on MDA production and hepar Tissue damages

**Table 3.** Effect of formalin exposure through the feeding diet on MDA Production

Formaldehid treatment	MDA ( µg/ml )					avara ge	MDA producti on (percent )
	1	2	3	4	5		
Kontrol (0 ppm)	5,31	5,47	5,41	5,29	5,30	5,36 <sup>a</sup>	1 3.40%
25 ppm	18,61	19,05	18,59	18,42	18,76	18,67 <sup>b</sup>	47.31%
50 ppm	29,59	29,29	29,25	29,70	29,60	29,49 <sup>c</sup>	74.73%
75 ppm	35,26	35,43	35,26	35,27	35,41	35,33 <sup>d</sup>	89.56 %
100 ppm	39,63	39,43	39,29	39,51	39,46	39,46 <sup>e</sup>	100 %



**Figure 1.** Effect of formaldehyde exposure on histopathology of rats hepar HE (400x)  
a.Rats hepar control c. Rats Hepar 50 ppm  
b.Rats hepar 25 ppm formalin d. Rats hepar 75 ppm

Data from **Table 3**, and **Figure 1**, Show that increasing treatment of doses level formaldehyde treatment lead to increasing production of malonyldialdehyde (MDA). MDA is substance as a result of oxidatif process of phospholipids oxidation of cell membrane and a parameter of cell membrane damages. Formaldehyde is a reactive compound, its easy to react to nucleophilic groups, particularly to NH<sub>2</sub> group of protein (enzyme system), that can effect on decreasing specific activity of enzyme, particularly of oxidative phosphorylation system of cytochrome P<sub>450</sub> disturbed. (Lee, 2003; and Bray 2005). Overall of these reactions will affect to acidosis condition of the cells and tissue, and tendency to over production of reactive oxygen species (ROS) and free radical substances, as a result of disturbed of Beta oxidation cycle. All of them will affect to decrease ATP production and to lead dead of cells as necrosis dead cells (Lee, 2003; Bray 2005).

Over production of ROS and free radical substances will affect of both to cell membrane damages, ion Ca<sup>++</sup> in cytosol, and stimulated inflammation of the cells and tissue of hepar, that characterized by generation of nitrooxide radicals (NO·), and its will react with radical superoxide (O<sub>2</sub>·) to

form peroxynitrite radical, that have more toxic potent. Formaldehyde exposure through the feeding diet in short period time will effect on stress oxidative and oxidative damages and reducing body immune, in long periods will generative of fifty degenerative disease affect by formaldehyde as a sources of ROS and free radical for instance are: Hepatitis, cataract genesis, Age pigment, Heart attack, Arthritis, Asthma, stroke, cancer, Atherosclerosis, periodontitis, and soon

### Conclusion

The overall of the research can be concluded that formalin exposure through the feeding diet of rats (*Rattus norvegicus*) affect decreasing highly significantly ( $P < 0,01$ ) on antioxidant enzymatic (SOD and GSH) and increasing oxidative damage (MDA) of liver tissue of rat (*Rattus norvegicus*).

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