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Hot Oil Exposure on Styrofoam and Black Crackle Bag Effect on Malondialdehyde MDA Level of White Rat (*Rattus norvegicus*) Wistar Strain

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ABSTRACT

This study purposed to determine the effect of hot oil exposure on styrofoam and black plastic bag on MDA levels in rats. The study was purely experimental, a sample of 28 Wistar rats, weight ±200gram, age 3 months, divided into 4 groups, treatment was given for 28 days. Negative control was given feed and drink; styrofoam group was given feed, drink, oil on styrofoam; the black plastic group was given feed, drink, oil on black plastic; positive control was given feed, drink, toluene mixed oil. On 14th and 28th-day blood was taken from the tail and examined for plasma MDA by spectrophotometer λ=532nm. One-way ANOVA shows there is an effect of hot oil exposure on styrofoam and black plastic on MDA levels on the 14th and 28th days (p=0.000). Post hoc showed the styrofoam group had a significant increase in MDA levels on 14th (p=0.003) and 28th (p=0.043) compared to negative controls, the black plastic group as well on 14th (p=0.000) and 28th (p=0.000). MDA levels between styrofoam and black plastic groups significantly different on 14th (p=0.012), but not on 28th (p=0.340). There was a significant MDA increase in the styrofoam group between 14th and 28th days (p=0.009), and in the black plastic group (p=0.031). This shows that hot oil exposure on styrofoam and black plastic cause cell oxidative stress.

Keyword: Black Plastic Bag, MDA, Oxidative Stress, Styrofoam

ABSTRAK

Penelitian bertujuan mengetahui pengaruh paparan minyak panas pada stirofoam dan kresek hitam terhadap kadar MDA. Penelitian merupakan eksperimental murni, sampel 28 ekor tikus putih galur Wistar, berat badan ±200gram, usia 3 bulan, dibagi menjadi 4 kelompok, perlakuan selama 28 hari. Kontrol negatif diberi pakan biasa dan air minum; kelompok stirofoam diberi pakan, minum, dan minyak pada stirofoam; kelompok kresek hitam diberi pakan, minum, dan minyak pada kresek hitam; kontrol positif diberi pakan, minum, dan minyak dicampur toluene. Mengambil darah dari vena ekor pada hari ke-14 dan ke-28 lalu memeriksa MDA plasma dengan spektrofotometer λ=532 nm. Hasil One-way ANOVA menunjukkan peningkatan MDA kelompok stirofoam dan kresek hitam hari ke-14 dan ke-28 (p=0.000). Uji post hoc menunjukkan MDA kelompok stirofoam meningkat signifikan pada hari ke-14 (p=0.003) dan ke-28 (p=0.043) dibanding kontrol negatif, begitu juga kelompok kresek hitam hari ke-14 (p=0.000) dan ke-28 (p=0.000). Kadar MDA antara kelompok stirofoam dan kresek hitam berbeda signifikan pada hari ke-14 (p=0.012), tetapi tidak pada hari ke-28 (p=0.340). MDA berbeda bermakna antara hari ke-14 dan ke-28 pada kelompok stirofoam (p=0.009) dan kelompok kresek hitam (p=0.031). Hal ini menunjukkan paparan minyak panas pada stirofoam dan kresek hitam dapat menimbulkan stres oksidatif sel.

Kata Kunci: Kresek Hitam, MDA, Stirofoam, Stres Oksidatif

INTRODUCTION

Plastics have lots of function, one of which is as a food container, like styrofoam, Tupperware, and mica. Plastic as a container for high temperature food could cause molecule migration into the

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food. Migration opportunities increase with increased in food emperature.[1]

Styrofoam products these days could befound as electronics container,fruit container,and food container.Data from Yogyakarta's Environmental Services[2] shows that among 350ton per day,there is about 25.83%styrofoam waste. Styrene migration from styrofoam occurs more in hot temperature and oily food. Styrene particles thatenter gonna be oxydated in cytochrome p450 into *7,8-oxide styrene*, then get metabolized furthermore to become mandelac acid, phenylglycolylic, and hipurate,or conjugated with glutathione to br phenylhydroxi-mercapurate. This conjugation will decrease*glutathione* (GSH) that makes cell membrane susceptible to damage and make end product whch is malondyaldehide (MDA).[3]

Oxide-7,8-styrene, intermediate molecules that can bind covalently with DNA,potential to induce genotoxic effect.[4] International Agency for Research on Cancer[5] classify oxide styrene as probable human carcinogen (Group 2A). Classification, Labelling, and Packaging Europe Regulation[6] classify into group 1B (considered have carsinogenic effect to human mosly by animal evidence). A study in 72.292 workers A Denmark company that produces plastics reported that there was increase case ratio of Hodgkin lymphoma,myeloid leukemia,and nasal cavity and sinus cancer,but nit consistent with exposure andduration of work.[7]

Crackle bag is one type of plastics to bring goods, but recently be used as a food container. Caution not to use crackle bag into direct contact with food was mentioned by Indonesian Food and Drugs Administrator (BPOM) in 2009.[8] Indonesia uses more than 1 million pieces of crackleper minute. High use of plastic bagand low in recycling makes Indonesia to become plastic waste contributor number two.[9]

Plasticbag main compoments is polyethylene, added with dioxyphtalate (DOP)anddye. This bag can be recycled three times, with black crackle bag as the results. In recycling, plastic bag is cut,than added and dyed. DOP can migrate inti food if get in contact with hot temperature food. Phtalate will he hydrolyzed in intestine and makes radical compound.[10]

This monoester makes lipidpero-xidation and increase MDA, catalase (CAT), and dismutase superoxide (SOD), also with menyebabkan peroksidasi lipid decrease in*gluthatione peroxidase* (GP), and*glutathione S-transferase* (GST). Oxidative stressactivate *Peroxisome Proliferated-Activated Receptor* (PPAR) that then disturb activity regulated by it, those are cell metabolism, growth, and proliferation.Organs mostly disturb are liver,brain,and reproduction organ.[11]

Situation where there is high use of styrofoam and crackle bag, oxidative stress by plastics molecule migration make researchersinterested to find out more about hoit oil effect when placed on styrofoam and crackle bag.

METHOD

Study Design

Study is pure experimental.Design wasposttest *only control group design*).[12]

Tools and Ingredients

Equipment usedwere analytical balance, shelves where mice cage storage, mice cages with size p × l × t = 30 × 45 × 20cm along with powder cage (10 gr / cage / week), wire cover, place food and drink, gloves latex hands, masks, measuring cups, 150 ml volume black glass bottles, 3 mL disposable syringes, gastric sonde, scalpel, EDTA tubes. centrifuges, stopwatches, micropipets and tips, Eppendorf 3µL tubes, and spectrophotometers.

Ingredients used were bulk cooking oil as much as 2.8 liters; black plastic bag with a size of 14 × 27cm, weight 50mg;

food stirofoam with size p × l × t = 19 × 13 × 7cm, weight 5 gram; and toluene 42 mL.

The experimental animals were male Wistar rats (Rattus norvegicus) with ± 200 gram body weight, 12 weeks of age, healthy conditions obtained from the Center for Biological Interuniversity Research (PPAU-IH), Bandung Institute of Technology, which has collaborated with the Pharmacology and Therapy Laboratory of the Faculty Padjadjaran University Medicine with total of 28 rats. Rats were divided into four groups, namely the negative control group (K-), the stirofoam group (KS), the black crackle group (KKH), and the positive control (K +).

Procedure

Making Treatments Material

The cooking oil used is bulk cooking which is still new which is sold in Cibinong tradisional market. The heating process begins by entering ± 100 mL of cooking oil into the pan, then the pan is heated until it reaches a temperature of 100oC. After that, the oil is poured into a Styrofoam container and allowed to stand for 5 minutes. Then, the oil is stored in a black glass bottle and then given a name tag. The oil stored is the result of exposure to 100 mL of oil in a container producing sufficient volume, even excess to be given for 28 days to one mouse. This step is done 7 times to get enough amount to be given to 7 rats. Stirofoam material is replaced with black crackle to make the KKH group treatment material, while for (K +), it is made by mixing 1.5 mL of oil with 1.5 mL of toluene for 1 rat / day.

Rats Care and Treatments

Rats were placed in 4 cages with 7 cows each, fed and drank ad libitum. Get identification marks on the tail and back, then adapt for 1 week. After 1 week, the four groups began to be given the following treatment.

The negative control group, which is only given normal food and drink ad libitum, without being treated. The Stirofoam (KS) group was given food, drink and cooking oil as much as 3 ml / head / day that had been exposed to stirofoam. The Black Crackle Group (KKH) was given food, drink, and cooking oil as much as 3 ml / head / day which had been exposed to black crackle. Positive control group, given food, drink, and cooking oil as much as 3 ml / head / day mixed with toluene in a ratio of 1: 1.

Every day the rats are monitored to see the movement, changes in behavior, and whether or not the rat died.

Sample Taking, Processing, and MDA Examination

Blood collection on the 14th day and 28th day through rat veins through the tail as much as 2 mL and then placed in the EDTA tube. Measurements were made 14 days from the start of treatment, and between the first and second measurements, according to the Guidelines for Acute Toxicity Tests on experimental animals.[13]

The EDTA tube was centrifuged at 3000 rpm for 10 minutes, then taken as much as 0.5cc serum, added 200µL 20% TCA, 400µL of 0.67% thiobarbiturate solution (made freshly by diluting 0.67gram of thiobarbiturate powder in 100mL distilled water), heated to 0.67% thiobarbiturate solution. water bath for 10 minutes, temperature 95-100oC then cool for 10 minutes. Supernatant is taken then put into a tube and added to 3cc aquabides. Calibration is done by placing the tube containing aquabides into the cuvette. After that, the tube containing the supernatant was placed into a cuvette, the absorption value was read at a wavelength of 532nm, then MDA levels were calculated using a standard curve.

DataAnalysis

MDA levels were analyzed using the One-Way ANOVA test. If there are significant differences, see different

groups through the Bonferroni post hoc test using SPSS 22 for Windows.

RESULT

These are MDA averages on 14th (table 1) and 28th day (table 2).

Table 1. Averages on 14th day

Group	Average±SD(µM)
Negative Control	1.45±0.32
Styrofoam Group	2.49±0.06
Crackle bag group	3.39±0.61
Positive control	3.55±0.68

Table 2. MDA average on 28th day

Group	Average±SD(µM)
Negative Control	1.70±0.39
Styrofoam Group	3.53±0.77
Crackle bag group	4.77±1.36
Positive control	6.00±1.67

Table 1 shows the mean MDA levels increased in the styrofoam, black crackle, and positive controls compared to negative controls. Table 2 shows the mean MDA levels increased in the styrofoam, black crackle, and positive controls compared to negative controls. MDA levels of the four groups increased compared to the 14th day.

One Way ANOVA and Post-Hoc Bonferroni Test Results

One Way ANOVA of MDA level on 14th and 28th day is in table 3.

Table 3. One way ANOVA Test result of MDA Level

MDA	Sig
MDAon 14th Day	0.000
MDAon 28th Day	0.000

One-Way ANOVA significance of 0,000 and 0,000 shows that there is a significant difference in mean MDA levels between groups on the 14th and 28th days. The mean difference between the four groups was seen through a post hoc test with the results shown in tables 4 and 5.

Table 4. Post-hoc Bonferroni on MDA Level Day-14

Group	Compared With	Sig.
Negative Control	Positive Control	0
	StyrofoamGroup	0.003
	Crackle Bag Group	0
Positive Group	StyrofoamGroup	0.002
	Crackle Bag Group	1
Styrofoam Group	Crackle Bag Group	0.012

Table 4 shows the negative control group had a significant difference in MDA levels in the styrofoam group (p = 0.003) and the black crackle group (p = 0.000). The positive control group had a significance of 0.002 against the styrofoam group which meant there was a significant difference, but not significantly to the black crackle group (p = 1,000). MDA levels between the styrofoam group and the black crackle group were significantly different, expressed with a significance value of 0.012.

Table 5. Post hoc Bonferroni of MDA Level Day-28

Group	Compared With	Sig.
Negative Control	Positive Control	0
	Styrofoam Group	0.043
	Crackle Bag Group	0
Positive Control	Styrofoam Group	0.003
	Crackle Bag Group	0.349
Styrofoam Group	Crackle Bag Group	0.34

The post hoc test results between the four 28th day groups listed in Table 5, showed the negative control group had significance of 0.043 and 0.000 for the stirofoam group and the black crackle group, which means there was a significant difference between the negative control group with the stirofoam group and the black crackle group.

The positive control group had a significance of 0.003 against the styrofoam group which meant there was a significant difference, but not significantly to the black crackle group (p = 0.349). MDA levels between the styrofoam group and the black crackle group were not significantly different (p = 0.340).

T Dependent Test Result

T dependent test result netween 14th and 28th day is in table 6.

Table 6. T dependent Test Result Between 14th and 28th Day

Group	Sig.
Negative Control	0.019
Positive Control	0.007
Styrofoam Group	0.009
Crackle Bag Group	0.031

DISCUSSION

Normal MDA levels in white Wistar rats according to Wulandari's study[14] amounted to 0.2202 ± 0.04 µM. The MDA level of the negative control group in the study by the authors was 1.45 ± 0.32 µM (table 1) on the 14th day. These different levels (greater than normal) can be caused by older rats' age.

MDA levels of negative controls on the 28th day were 1.70 ± 0.39 µM (table 2). This level increases from day 14, although no treatment is given which causes oxidative stress. This can occur due to stress in mice, such as stress due to daily treatment and aging, so endogenous antioxidants such as superoxide dismutase and glutathione decrease.

The average increase in MDA levels was found in the stirofoam group, black crackle (table 1) indicating the presence of oxidative stress that occurs in cells. This is influenced by the migration of styrene and dioxylphthalate molecules, as well as differences in the ability of these molecules to cause oxidative stress. This study did not measure levels of migration, but previous studies by Alin[15] showed that there was styrene migration due to exposure to styrofoam to heat. This result is the opposite of research conducted by the Center for Food Safety, Hong Kong, namely styrene migration only on 2 containers of instant noodles and 5 capsules of instant noodles. This can happen if the instant noodle stirofoam manufacturing plant has measured and set a threshold to prevent dangerous migration. Research by Moreira[16] shows that there is phthalate migration due to plastic exposure to heat. Mattia's study cited in Tatjana[17] in the form of administering various doses of toluene resulted in the formation of ROS which is directly proportional to the dose. The increase in MDA levels in this study is in accordance with previous studies.

After the intervention continued for 28 days, the MDA levels of the styrofoam, black crackle, and positive control groups increased (table 2). This situation shows that during the time span between the 14th and 28th day, lipid peroxidation occurred continuously. Exposure to styrene and dioxylphthalates continues so that cell membrane damage is more extensive. The accumulation of MDA that has been formed for 14 days will further trigger peroxidation in the surrounding area so that the membrane damage becomes even more extensive, contributing to the MDA levels continue to increase.

ANOVA test results showed there was an effect of exposure to hot oil on stirofoam and black crackle on MDA levels (p=0.000) on the 14th and 28th days

(table 3). The MDA levels of the stirofoam group increased significantly compared to the negative control (p = 0.003) day 14 (table 4), and p = 0.043 day 28 (table 5). Based on the theory, styrene can reduce the levels of gluthatione so that cells are susceptible to free radicals. Research by Haghighat[18] compared MDA levels due to styrene exposure compared to negative controls, showing a significant increase. The results of this study are consistent with the theory and supported by previous research.

The MDA level of the styrofoam group was still significantly lower than the positive control (p = 0.002) day 14 (table 4) and p = 0.003 day 28 (table 5). There are no studies comparing the effects of styrene molecules on toluene. The MDA levels of the styrofoam group are lower than the positive control, indicating that toluene does have a very strong lipid peroxidation effect, even based on Mattia's research[17] it can also cause a decrease in intracellular antioxidants. There are no studies comparing MDA levels due to styrene compared to phthalates.

Table 4 shows that there are differences in plasma MDA levels between the administration of hot oil exposed to styrofoam and black crackle after 14 days (p = 0.012), indicating that the stress caused by styrene oxide is lower than that due to dioxylphthalate. Differences in plasma MDA levels in the styrofoam and black crackle groups were present after 14 days of exposure, but not after 28 days (p = 0.340) (table 5). The effects of styrene tend to get heavier after exposure due to longer accumulation of styrene in adipose tissue which can increase the weight of the liver, kidneys, and growth disorders [19], while the effects of phthalates occur tend to be faster, which is about 24 hours after exposure.[20] Therefore, the authors' research results are in accordance with previous studies.

Significant improvement in MDA of the black crackle group compared to negative control was found on day 14 (p = 0,000) (table 4) and day 28 (p = 0,000) (table 5). Research by Asghari and Mattia proves that phthalate ROS formation, DNA damage, lipid peroxidation, and disturbing antioxidants.[11,17] These lipid peroxidations cause MDA levels to rise. The increase in MDA in this study is in accordance with previous studies.

MDA levels that were not significantly different between black crackle groups with positive controls on day 14 (p=1,000) (table 4) and day 28 (p=0.043) (table 5) showed the effect of lipid peroxidation due to phthalates was almost the same with effects due to toluene, but there is no research related to this.

A significant increase in plasma MDA levels in the stirofoam group was found between days 14 and 28 (p = 0.009) (table 6). These results indicate that the stress due to styrene oxide from styrofoam initially was not large, but the effect accumulated after continuous exposure, causing a significant increase after 28 days. There has been no research on styrene exposure to MDA levels. Bloemen[19] mentions that repeated exposure to styrene in mice causes accumulation of styrene in adipose tissue which can increase weight of the liver, kidneys, and growth and development disorders. Thus, a significant increase in MDA on the 28th day compared to the 14th day according to previous research.

There was a significant increase in plasma MDA levels in the black crackle group between days 14 and 28 (p = 0.009) (table 6). This shows the effects of lipid peroxidation occur continuously. There are no previous studies that support this. Research by Asghari[11] did not compare the duration of exposure to MDA levels.

CONCLUSION

Based on results and discussions,can be infered that hot oil exposureto styrofoam can increase plasma MDA levelswhen checked on 14th day (p=0.003) and 28th day (p=0.000) compared with

negative control. Hotoil exposure to crackle bag can increaseplasma MDA levels chen checked on 14th day(p=0.043)and 28th day (p=0.000). Plasma MDA levels significantly differbetween styrofoam group, and crackle bag group on 14th day (p=0.012) but not on 28th day (p=0.340)

SUGGESTION

Based on this research,there are some suggestions to be considered, such as:

a. For societies
   Crackle bag should not be used for direct contact with food. Styrofom can be used as a food container, but not in hot temperature. Avoid wraping hot food withplastics.
b. For another researchers
   Styrene molecules migration level from styrofoam and phtalate from crackle bag should be tested to know more about the exact amount of molecules do migrate. Also should be done research about another plastics type as food container and its effects on health.

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