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The Effect of Feeding Non-Antioxidant Feed on The Degree of Dysplasia in Formaldehyde-Induced Wistar Rats

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Abstract: Nasopharyngeal dysplasia is a condition of abnormal cellular growth that occurs due to the failure of nasopharyngeal epithelial cells in carrying out cellular adaptation. This condition can occur due to the continuous inhalation of toxic compounds, such as formaldehyde, which has genotoxic and cytotoxic effects that can induce cellular damage. One of the compound activity associated with reducing risk of dysplasia is antioxidant activity. Therefore, it is important to know whether the presence of antioxidant activity affects the occurrence of dysplasia. This study is an experimental study with a post-test only control group design which aims to determine the effect of Non-Antioxidant Feed on the occurrence of nasopharyngeal dysplasia. Testing of the antioxidant activity in feed was carried out using the DPPH assay. There were two groups of experimental animals including the treatment group (fed with Non-Antioxidant Feed) and the control group (fed with SB 11). All animals in each group were induced with 40 ppm formaldehyde by inhalation for 8 weeks and then euthanized using chloroform inhalation for nasopharyngeal tissue extraction. The results of the histopathological examination of the two groups were then statistically tested using the Mann-Whitney U test and obtained a value of $P<0.05$ ($P=0.006$), which means that there are effect of feeding Non-Antioxidants Feed on the degree of dysplasia in formaldehyde-induced wistar rats. The results can be associated with the ingredients contained in the feed given, differences in genetic polymorphisms of each subjects, and effect of body weight variations of the rats caused by randomization.

Keywords: Antioxidant, dysplasia, feed, formaldehyde, nasopharynx.

Introduction

Malignancy is disease that contributes to the high mortality rate worldwide. Based on data of cancer incidence and mortality by the International Agency for Research on Cancer (IARC), it is estimated that there will be 19.3 million new cases of malignancy and nearly 10 million cases of death from malignancy in 2020 (Sung et al., 2021). About 30% of malignancies that occur in developing countries such as countries in Asia and North Africa are caused by human papillomavirus (HPV) infection and hepatitis with one type of malignancy that

often occurs is squamous cell malignancy of the head and neck (World Health Organization, 2022).

Nasopharyngeal dysplasia is one of head and neck squamous cell malignancy phases in the form of cellular abnormalities that occur due to the failure of cells to adapt to their environment due to continuous exposure to damaging stimuli. Continuous exposure to damaging stimuli can result in cells developing cellular atypia characterized by abnormalities such as increased replication rates. Cellular atypia often causes maturation failure in cells so that the final product is not appropriate. Cellular atypia that occurs in cells can be transient or persistent and can progress to dysplasia. Nasopharyngeal dysplasia can develop into invasive carcinomas such as nasopharyngeal carcinoma (Johnson et al., 2020; Young et al., 2017).

Dysplasia in the nasopharynx can be caused by inhalation of carcinogenic substances. One substance that is closely related to the occurrence of nasopharyngeal dysplasia is formaldehyde. Formaldehyde (CH_2O or H_2CO) is a carcinogenic substance that is commonly found in everyday life such as smoke from combustion activities, various paint products, or preservative liquids such as formalin (Swenberg et al., 2013). Formaldehyde can have toxic effects if inhaled, direct contact with the skin or eyes, and if the formaldehyde is ingested into the body (Tesfaye et al., 2020). The International Agency for Research on Cancer (IARC) has classified formaldehyde as carcinogenic to humans due to its ability to cause malignancies in the nasopharynx, sinonasal, and leukemia. Malignant cell formation can arise due to the cytotoxicity and genotoxicity properties of formaldehyde (Leso et al., 2020).

In addition to the inhalation of carcinogenic substances, malignancy can also be triggered by the consumption of foods that contain carcinogens. Based on research by Kamal et al. (2022), carcinogenic substances that we often encounter in food such as residual pesticides on food ingredients; pyrrolizidine alkaloids that are commonly found in drinks such as tea; hydrazine in mushrooms; safrole and alkenylbenzene in food flavorings; and mycotoxin content produced by fungi in stale food (Kamal et al., 2022).

One of the substances associated with a reduced risk of malignancy is antioxidant activity (Duthie et al., 2017). This correlates with the activity of antioxidants in delaying, inhibiting, or slowing down the oxidation reactions of other molecules. Antioxidants can neutralize uncontrolled free radicals by donating their electrons so that the free radicals can be controlled and the capacity of free radicals to damage cells can be reduced (Setiawan et al., 2021). However, the exact relationship of the presence of antioxidant activity to cell malignancy is still controversial (Mulia et al., 2016; Rytsyk et al., 2020; Sayin et al., 2014; Zhang et al., 2021)

Research that examines the relationship between formaldehyde exposure and the occurrence of dysplasia in the nasopharynx is mostly in the form of experimental research using animals such as research by Bbosa et al. (2013) and Susilawati et al. (2022). However, various studies that have been conducted previously have not reviewed the activity of the feed content given to the experimental animals used and its relationship with the occurrence of dysplasia in research subjects where it can affect the final results of the study. Based on this background, the authors became interested in further investigating the relationship between the presence of antioxidant activity in the feed of animal models and cell progression by conducting experimental research on the effect of feeding Non-Antioxidants Feed on nasopharyngeal dysplasia progression.

Material and Method

Time and place

This study is an experimental study with a post test only control group design conducted from March-April 2023. Formaldehyde dilution and preparation of Non-antioxidants feed were carried out at the Pharmacochemistry Laboratory of the Pharmacy Study Program, Faculty of Medicine, Mataram University. Formaldehyde induction and feeding of Wistar rats (*Rattus norvegicus)* were carried out at the Drug Testing Laboratory of the Pharmacy Study Program, Faculty of Medicine, Mataram University. The antioxidant activity assay was carried out at the Analytical Laboratory, Faculty of Mathematics

and Natural Sciences, Mataram University. Histopathology examination was carried out at the Anatomical Pathology Laboratory, University of Mataram Hospital.

Population and sample

The samples used in this study consisted of 12 male Wistar rats (*Rattus norvegicus*) aged 8-12 weeks weighing 100-200 grams. The independent variable in this study is Non-Antioxidants Feed, while the dependent variable in this study is the degree of dysplasia. Before the study began, rats were acclimatized for 1 week at the Drug Testing Laboratory of the Pharmacy Study Program, Faculty of Medicine, Mataram University. After that, the rats were then randomized and divided into two groups, namely the treatment group and the control group (each group consisted of 6 rats). The rats were placed in a specially designed cage to induce dysplasia effectively and economically which had ventilation on both sides of the cage with a size of 28 cm x 22.5 cm x 10 cm by Wedayani et al. (2023). After being placed into the appropriate cages, the rats were weighed using an analytical scale to obtain specific body weight data.

In this study, rats in the treatment group were fed with Non-Antioxidants Feed according to the recipe "Laboratory Animal Feed Without Antioxidant Activity as Animal Feed for the Preparation of Animal Models in Health Studies" by Putri et al. (2023) which has been copyrighted with HAKI record number 000476100 and rats in the control group were fed with SB 11 feed due to its nutritional content. Before being given, the feed was tested for antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. All rats in each group were induced with 10% formaldehyde at 40 ppm dose by inhalation for 6 hours everyday, for 8 weeks. This method was designed as a modification of the study by Susilawati et al., (2022).

After 8 weeks, all rats were euthanized using chloroform inhalation and then terminated for nasopharyngeal tissue extraction. The ethics of this study have been reviewed and have obtained permission from the Ethics Committee, Faculty of Medicine, University of Mataram (087/UN18.F8/ETIK/2023). Samples were placed in tubes with formalin solution for preservation and sent to the Anatomical Pathology Laboratory, Faculty of Medicine, University of Mataram. Samples were then fixed, dehydrated, sectioned, rehydrated, and stained with Hematoxylin-Eosin stain. The tissue slides of the samples were then examined to determine the degree of dysplasia. In this study, the degree of dysplasia was categorized as no dysplasia, mild dysplasia, moderate dysplasia, and severe dysplasia.

Statistical analysis

The histopathological data obtained were analyzed statistically using the Statistical Package for the Social Sciences (SPSS) program. The statistical assay was performed using the Mann-Whitney U test, adjusted for the number of data groups, unpaired data type, and ordinal categorical data scale.

Result dan Discussion

Antioxidant activity of non-antioxidants feed

The antioxidant activity of Non-Antioxidants Feed was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. DPPH assay is one of the in vitro methods to test antioxidant activities using the principle of antioxidant interaction with DPPH (1,1-diphenyl-2-picrylhydrazyl) as a free radical by examining the IC50 value (Marjoni & Zulfisa, 2017).

Table 1. Data from antioxidant activity test using DPPH method

Concentration (ppm)	Absorbance(λ)	Blank	Inhibiton Percentage (%)
100000	0,3186	0,7847	59,398496
80000	0,3731	0,7847	52,453166
60000	0,4502	0,7847	42,627755
40000	0,5838	0,7847	25,602140
20000	0,6625	0,7847	15,572830

DPPH assay consists of several processes, including the preparation of test samples of Non-Antioxidants Feed and preparation of DPPH stock solution, preparation of blank solution as control solution, preparation of the main sample solution, preparation of variation of concentration series of test solution (20.000 ppm, 40.000 ppm, 60.000 ppm, 80.000 ppm, and 100.000 ppm), mixing of test solution with DPPH, incubation of solution, observation of

color change in solution, and absorbance reading using ultraviolet visible spectrophotometer with maximum absorbance wavelength of 516 nm. The results of the blank absorbance data and the absorbance of the sample were then substituted into the formula of the percentage of DPPH radical inhibition to obtain data as in Table 1.

Results of qualitative and quantitative analysis of antioxidant activity in non-antioxidants feed

The data of antioxidant activity test results listed in Table 1 were analyzed qualitatively and quantitatively. Qualitative analysis was performed through observation of the color changes in the test solution and control solution during the DPPH test. Quantitative test is done by substituting the data into the regression equation that is made by using Microsoft Excel with the extract concentration as abscissa (X axis) and the percentage of inhibition as the ordinate (Y axis) so that the regression equation curve is obtained as shown in Figure 1.

After the regression equation curve is obtained, the value of 50 is substituted as the y value in the equation so that the final value of IC50 is 75,836.67µg/ml. From the qualitative and quantitative test results, it can be concluded that the feed in this study does not contain antioxidant activity as indicated by no color change in the qualitative test and IC50 value >500 µg/ml in the quantitative test (Arista, 2013; Munteanu & Apetrei, 2021)

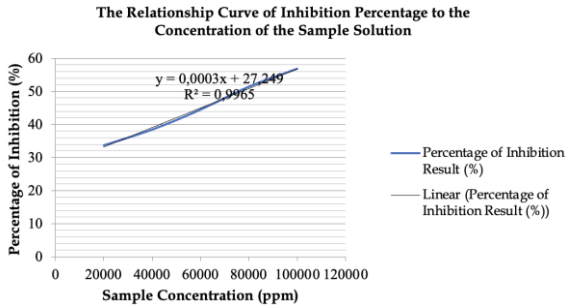

Figure 1. Relationship Curve between Percentage Inhibition and Sample Solution Concentration

Body weight data of rats before and after treatment

The selection of rats in each group was randomized. Rats were weighted before and after being treated for 8 weeks. Body weight data from weighing rats can be seen in Table 2.

Table 2. Body weight data of rats before treatment

Number of Rats	Control Group		Treatment Group	
	Before (g)	After (g)	Before (g)	After (g)
1	165	180	100,05	188,52
2	176	178	111	164,21
3	186,5	185	104,62	167,11
4	198,5	183	105,1	135,98
5	110	131	131,84	164,96
6	163,67	165	145,74	226,07

Histopathological analysis result of the nasopharyngeal tissue

Tissue samples were stained with Hematoxylin-Eosin staining and viewed under the microscope to see the degree of dysplasia. These are the result of nasopharyngeal tissues sample.

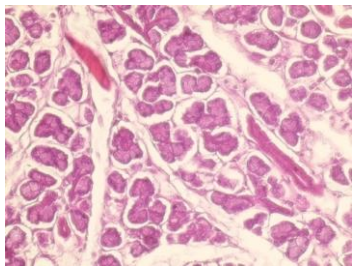

Figure 2. No dysplasia in the nasopharyngeal tissue of Wistar rats in the treatment group after 40 ppm formaldehyde induction for 8 weeks.

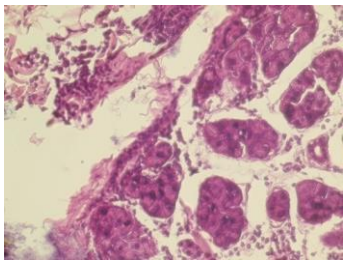

Figure 3. Mild dysplasia in nasopharyngeal tissue of Wistar rats in control group after 40 ppm formaldehyde induction for 8 weeks.

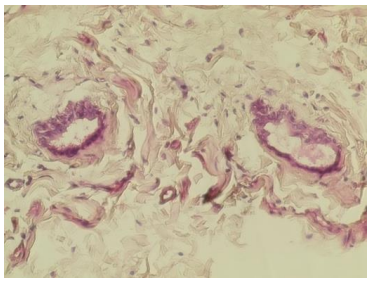

Figure 4. Moderate dysplasia in nasopharyngeal tissue of Wistar rats in control group after 40 ppm formaldehyde induction for 8 weeks.

Nasopharyngeal tissue degree of dysplasia

Histopathological examination of the nasopharynx of Wistar rats was performed at the Anatomical Pathology Laboratory of Mataram University Hospital. The stages of the histopathological examination process consist of making nasopharyngeal tissue preparations, staining using Hematoxylin-Eosin (H&E) staining, and histopathological examination using a digital microscope. Hematoxylin-eosin (H&E) staining was chosen because this stain is commonly used to identify changes in tissue morphology (Fischer et al., 2008). The results of the histopathological examination of the nasopharynx of Wistar rats in each group can be seen in Table 3.

Table 3. Histopathological examination results of nasopharyngeal tissues of rats in the treatment group and control group

Number of Rats	Degree of Dysplasia	
	Treatment Group	Control Group
1	No dysplasia	Moderate dysplasia
2	No dysplasia	Moderate dysplasia
3	No dysplasia	Moderate dysplasia
4	No dysplasia	Moderate dysplasia
5	No dysplasia	Mild dysplasia
6	No dysplasia	No dysplasia

All rats from each group managed to survive until the end of the study. Based on the results of histopathological examination as shown in Table 3, it was found that there were differences in the incidence of nasopharyngeal dysplasia of Wistar rats in the treatment and control groups. The results of histopathological examination of nasopharyngeal tissue of Wistar rats in the treatment group were all rats fed with Non-Antioxidants Feed did not have nasopharyngeal dysplasia.

Table 4. Frequency of research outcome variables

Variable	N (%)
Feed Type	
Non-Antioxidants Feed	6 (50.0%)
SB 11 feed	6 (50.0%)
Degree of Dysplasia	
No dysplasia	7 (58.3%)
Mild dysplasia	1 (08.3%)
Moderate dysplasia	4 (33.3%)
Severe dysplasia	0 (00.0%)

On the other hand, there were variations in the results of histopathology examination of nasopharyngeal tissues of Wistar rats in the control group with 4 rats having moderate degree of dysplasia, 1 rat having mild degree of dysplasia, and 1 rat having no dysplasia. The variable frequency of the overall study results is shown in Table 4 as follows.

Bivariate analysis of the histopathological examination findings

Based on the results of statistical analysis using the Mann Whitney-U Test outlined in table 5, the P-value is <0.05 (P = 0.006). The results of the P-Value in this study were significant because it is smaller than 0.05. From the statistical analysis data, it can be concluded that there is a significant effect of feeding Non-Antioxidants Feed on the incidence of dysplasia in Wistar rats.

Table 5. Mann-Whitney U statistical test

Degree of Dysplasia	Group		P-Value
	Treatment	Control	
ND	1 (16.7%)	1 (16.7%)	P<0,05 (P=0,006)
Mild	0 (0.0%)	1 (16.7%)	
Moderate	0 (0.0%)	4 (66,7%)	
Severe	0 (0.0%)	0 (0.0%)	
Total Sample	**6 (100.0%)**	**6 (100.0%)**	

*ND = No Dysplasia

Discussion

This study is a novelty study which similar research that examines the effect of feeding Non-Antioxidants Feed on the degree of dysplasia has never been published before. Data from statistical test analysis of the degree of dysplasia in the treatment and control groups showed that there are significant effects of feeding Non-Antioxidants Feed on the incidence of dysplasia in Wistar rats. Although significant results were obtained, the results of this study contradict the theory stated in the study by Poljsak, Šuput, dan Milisav (2013) dan Aggarwal et al., (2019) where the absence of antioxidant activity in food can accelerate cell proliferation in causing malignancy through accelerated accumulation of

Reactive Oxygen Species (ROS) in triggering oxidative stress, DNA damage, lipid peroxidation, and protein damage.

Table 6. Anti-malignancy activities of protein and enzyme content in egg white (Andersen, 2015)

Egg White Components	Mechanism
Cystatin	• Inhibits tumor-associated activity of intracellular cysteine proteases • Reduces the activity of cathepsins B and L which are associated with cancer cell invasion capabilities
Avidin	• Enhances the binding and persistence of TNF-α on tumor cells and increases the antitumor activity of TNF-α
Lysozyme	• Enhance immune system response and immune system activation or increase immunogenicity of body cells against tumors • Reduce tumor growth and prevent the formation of tumor metastases
Fosvitin	• Inhibits cancer cell growth • Protects DNA damage-Inhibits cancer cell growth • Protects DNA damage
Ig-Y	• Induces MCF7 cell death as one of the cancer inducing cells
Ovomucin	• Ovomucin β-subunit exhibits cytotoxicity in cultured tumor cells • Has antitumor activity that prevents swelling and neoangiogenesis of tumor cells
Ovotransferrin	• Inhibits proliferation of MCF-7 and HCT-116 cancer cells • Has cytotoxic effects to cancer cells
Ovalbumin	• Heat-denatured ovalbumin exhibits antimutagenic activity against Methylnitronitrosoguanidine (mutagen) in E. coli DNA repair system

No dysplasia in rats that fed Non-Antioxidants Feed may be influenced by the anti-malignancy activities in the egg white components of the rat feed given to the treatment group. Egg white consists of 54% ovalbumin, 12% ovotransferrin, 3.5% ovomusin, 3.4% lysozyme, and 0.5% avidin. These natural protein components can act as immunoprotective and antimicrobial agents through various mechanisms of inhibiting tumorigenesis and carcinogenesis (Andersen, 2015). In addition, there are also other proteins such as immunoglobulin Y, fosvitin, and avidin that can inhibit cell proliferation so as to prevent malignancy (Lee & Paik, 2019). The results of previous study about anti-malignancy activities of the egg white components can be seen in Table 6. Further research is required concerning feed containing egg white on the activity of the protein components in it and the effectiveness of these components in preventing malignancies.

Other than egg white components, evidence has also shown the involvement of aflatoxin in the development of various malignancies. The feed that given to the control group rats contained aflatoxin with a maximum amount of 50μg/kg written on the nutritional value information of the feed (PT. Charoen Pokphand Indonesia Tbk., n.d.). Aflatoxin is a type of mycotoxin produced by Aspergillus fungus species such as *Aspergillus flavus* and *Aspergillus parasiticus* that can cause aflatoxicosis in animals through ingestion and inhalation of aflatoxin particles (Bbosa et al., 2013; Shabeer et al., 2022). Chronic exposure to aflatoxins can cause chronic aflatoxicosis which has teratogenic, mutagenic, and carcinogenic effects through the genotoxic properties of aflatoxins which can damage DNA formation and induce the formation of DNA adducts (Bbosa et al., 2013).

In animal models, the chronic effects of aflatoxins can disrupt normal immune function by reducing phagocytic activity or reducing the number of T cells resulting in immunological suppression as well as causing modification of nutrients such as vitamin A or D in animal models resulting in nutritional deficiencies in the animals (Birnbaum et al., 1993; Doi & Uetsuka, 2011; Peraica et al., 1999; Wangikar et al., 2005; World Health Organization, 2000). Although the available evidence comes from chronic exposure to aflatoxin, this does not rule out a potentially permissive or inductive role that this substance may play in the dysplasia that occurs in this study.

In addition to the influence of the content contained in the composition of the feed given, the difference in the results of the study with the existing theory can be attributed to the difference in body weight of the rats in the two groups at the beginning of the study as a result of blind picking. Qualitatively, the average body weight of Wistar rats used in the control group was higher than in the treatment group. Based on a journal by Ghasemi et al., (2021) it was conclude that animal body weight can affect drug metabolism, gene expression, metabolic parameters, and other dependent variables measured in animal studies.

The age range of Wistar rats used in this study is quite far so that it can affect the results of the study where the age of the subject under study has an influence on the ability of cells to proliferate and the susceptibility of cells to malignancy (White et al., 2015). This can be used as a research limitation where future research is expected to homogenize the research population more optimally. Moreover, future studies may extend the study time to further assess the significance of this study where a similar experimental study examining the impact of formaldehyde on hyperplasia/ metaplasia/ dysplasia in cells was conducted for 26 weeks by Susilawati et al. (2022); 52 weeks by boyse et al. (1990); 13 weeks by oleh Nielsen, Larsen dan Wolkoff (2013); and 18 weeks by Golalipour et al., (2007) and Maronpot et al., (1986).

The difference in the results of histopathological examination of the nasopharynx in each rat can also be attributed to differences in genetic polymorphism in each living being where each living being has a different ability and length of time in responding to exposure to toxic substances such as carcinogens even though they come from the same species (Triwani and Saleh, 2015). This can be a limitation in research to be developed in future studies.

Conclusion

The results of this study showed a significant effect of feeding Non-Antioxidants Feed on the degree of dysplasia in formaldehyde-induced Wistar rats (*Rattus norvegicus*). This study contradicts the existing theory regarding the relationship between antioxidants and cell progressivity. This can be attributed to the content of the food given, the differences in genetic polymorphism of each subject, and the difference in body weight of the rats at the beginning of the study as a result of randomization. This study also has limitations in the form of too far age range in the study population. Future research is expected to extend the time period of the study to increase the significance of the results.

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References

Andersen, C. J. (2015). Bioactive egg components and inflammation. *Nutrients*, *7*(9), 7889–7913. DOI: https://doi.org/10.3390/nu7095372

Arista, M. (2013). Aktivitas Antioksidan Ekstrak Etanol 80% Dan 96% Daun Katuk (Sauropus androgynus (L.) Merr. *Jurnal Ilmiah Mahasiswa Universitas Surabaya*, *2*(2).

Bbosa, G. S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W. W., & Kyegombe, D. B. (2013). Review of the Biological and Health Effects of Aflatoxins on Body Organs and Body Systems. In *Aflatoxins - Recent Advances and Future Prospects* (Vol. 12). InTech. DOI: https://doi.org/http://dx.doi.org/10.5772/51201

Birnbaum, L. S., Anisimov, V. N., United Nations Environment Programme., International Labour Organisation., World Health Organization., & International Program on Chemical Safety. (1993). *Principles for evaluating chemical effects on the aged population*. World Health Organization.

Boysen, M., Zadig, E., Digernes, V., Abeler, V., & Reith, A. (1990). Nasal mucosa in workers exposed to formaldehyde: a pilot study. In *British Journal of Industrial Medicine* (Vol. 47).

Doi, K., & Uetsuka, K. (2011). Mechanism of Mycotoxin-Induced Neurotoxicity through Oxidative Stress-Associated Pathways. *International Journal of Molecular Sciences*, *12*. DOI: https://doi.org/10.3390/ijms12085213

Duthie, S. J., Duthie, G. G., Russell, W. R., Kyle, J. A. M., Macdiarmid, J. I., Rungapamestry, V., Stephen, S., Megias-Baeza, C., Kaniewska, J. J., Shaw, L., Milne, L., Bremner, D., Ross, K., Morrice, P., Pirie, L. P., Horgan, G., & Bestwick, C. S. (2017). Effect of increasing fruit and vegetable intake by dietary intervention on nutritional biomarkers and attitudes to dietary change: a randomised trial. *European Journal of Nutrition*, *57*(5), 1855–1872. DOI: ttps://doi.org/10.1007/s00394-017-1469-0

Fischer, A. H., Jacobson, K. A., Rose, J., & Zeller, R. (2008). Hematoxylin and eosin staining of tissue and cell sections. *CSH Protocol*.

Ghasemi, A., Jeddi, S., & Kashfi, K. (2021). The Laboratory Rat: Age And Body Weight Matter. In *Excli Journal* (Vol. 20, pp. 1431–1445). Leibniz Research Centre for Working Environment and Human Factors. DOI: https://doi.org/10.17179/excli2021-4072

Golalipour, M. J., Azarhoush, R., Ghafari, S., Gharravi, A. M., Fazeli, S. A., & Davarian, A. (2007). Formaldehyde exposure induces histopathological and morphometric changes in the rat testis. *Folia Morphol*, *66*(3), 167–171. URL: www.fm.viamedica.pl

Johnson, D. E., Burtness, B., Leemans, C. R., Lui, V. W. Y., Bauman, J. E., & Grandis, J. R. (2020). Head and Neck Squamous Cell Carcinoma. *Nature Reviews*, *6*(92). DOI: https://doi.org/10.1038/s41572-020-00224-3

Kamal, N., Ilowefah, M. A., Hilles, A. R., Anua, N. A., Awin, T., Alshwyeh, H. A., Aldosary, S. K., Jambocus, N. G. S., Alosaimi, A. A., Rahman, A., Mahmood, S., & Mediani, A. (2022). Genesis and Mechanism of Some Cancer Types and an Overview on the Role of Diet and Nutrition in Cancer Prevention. *Molecules*, 1–27.

Lee, J. H., & Paik, H. D. (2019). Anticancer and immunomodulatory activity of egg proteins and peptides: a review. *Poultry Science*, *98*(12), 6505–6516. DOI: https://doi.org/10.3382/ps/pez381

Leso, V., Macrini, M. C., Russo, F., & Iavicoli, R. (2020). Formaldehyde Exposure and Epigenetic Effects: A Systematic Review. *Multidisciplinary Digital Publishing Institute (MDPI)*, *10*.

Marjoni, M., & Zulfisa, A. (2017). Antioxidant Activity of Methanol Extract/Fractions of Senggani Leaves (Melastoma candidum D. Don). *Pharmaceutica Analytica Acta*, *08*(08). DOI: https://doi.org/10.4172/2153-2435.1000557

Maronpot, R. R., Miller, R. A., Clarke, W. J., Westerberg, R. B., Decker, J. R., & Moss, O. R. (1986). Toxicity Of Formaldehyde Vapor In B6c3f1 Mice Exposed For 13 Weeks. In *Toxicology* (Vol. 41).

Mulia, K., Hasan, A. E. Z., & Suryani. (2016). Total Phenolic, Anticancer and Antioxidant Activity of Ethanol Extract of Piper retrofractum Vahl from Pamekasan and Karang Asem. *Current Biochemistry*, *3*(2), 80–90. URL: http://biokimia.ipb.ac.id

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, *22*(7). DOI: https://doi.org/10.3390/ijms22073380

Nielsen, G. D., Larsen, S. T., & Wolkoff, P. (2013). Recent trend in risk assessment of formaldehyde exposures from indoor air. In *Archives of Toxicology* (Vol. 87, Issue 1, pp. 73–98). DOI: https://doi.org/10.1007/s00204-012-0975-3

Peraica, M., Radic, B., Lucic, A., & Pavlovic, & M. (1999). Toxic effects of mycotoxins in humans. *Bulletin of the World Health Organization*, *77*.

Poljsak, B., Šuput, D., & Milisav, I. (2013). Achieving the balance between ROS and antioxidants: When to use the synthetic

antioxidants. *Oxidative Medicine and Cellular Longevity*. DOI: https://doi.org/10.1155/2013/956792

PT. Charoen Pokphand Indonesia Tbk. (n.d.). *SB 11*. PT. Charoen Pokphand Indonesia Tbk.

Putri, N. P. A. N. D. C., Wedayani, A. A. A. N., Punagi, A. Q., Pieter, N. A. L., Cangara, M. H., Kadriyan, H., Susilawati, N. K., Hidayat, L. H., Fauzan, M., Makbul, I. H., Wiguna, I. M. D., Abdiman, I. M. T., Nugraha, G. R. H., Yassa, I. W. R. W., Kusuma, I. A. J., Malaka, R., & Pratiwi, S. (2023). *Pakan Hewan Laboratorium Tanpa Aktivitas Antioksidan sebagai Pakan Hewan Untuk Pembuatan Hewan Model pada Studi Kesehatan* (Patent No. 000476100). URL: www.tcpdf.org

Rytsyk, O., Soroka, Y., Shepet, I., Vivchar, Z., Andriichuk, I., Lykhatskyi, P., Fira, L., Nebesna, Z., Kramar, S., & Lisnychuk, N. (2020). Experimental Evaluation of the Effectiveness of Resveratrol as an Antioxidant in Colon Cancer Prevention. *SAGE Journal*, *15*(6), 1–10.

Sayin, V. I., Ibrahim, M. X., Larsson, E., Nilsson, J. A., Lindahl, P., & Bergo, M. O. (2014). Antioxidants Accelerate Lung Cancer Progression in Mice. *Science Translational Medicine*, *6*(221). URL: www.ScienceTranslationalMedicine.org

Setiawan, V., Phangestu, S., Grace Soetikno, A., Arianti, A., & Kohar, I. (2021). Rapid screening analysis of antioxidant activities in green tea products using DPPH and FRAP. *Pharmaceutical Journal of Indonesia*, *7*(1), 9–14. DOI: https://doi.org/10.21776/ub.pji.2021.007.01.2

Shabeer, S., Asad, S., Jamal, A., & Ali, A. (2022). Aflatoxin Contamination, Its Impact and Management Strategies: An Updated Review. In *Toxins* (Vol. 14, Issue 5). MDPI. DOI: https://doi.org/10.3390/toxins14050307

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. DOI: https://doi.org/10.3322/caac.21660

Susilawati, N. K., Yasa, I. W. P. S., Suardana, W., Maliawan, S., Jawi, I. M., Romdhoni, A. C., Kadriyan, H., Putra, I. W. G. A. E., & Arijana, I. G. K. N. (2022). Purple Sweet Potato Reduces Malondialdehyde and TNF-a, Increases p53, and Protects Histopathological Appearance in Formaldehyde-induced Nasopharyngeal Carcinoma Rats. *Indonesian Biomedical Journal*, *14*(2), 211–217. DOI: https://doi.org/10.18585/inabj.v14i2.1906

Swenberg, J. A., Moeller, B. C., Lu, K., Rager, J. E., Fry, R. C., & Starr, T. B. (2013). Formaldehyde Carcinogenicity Research: 30 Years and Counting for Mode of Action, Epidemiology, and Cancer Risk Assessment. *Toxicologic Pathology*, *41*(2), 181–189. DOI: https://doi.org/10.1177/0192623312466459

Tesfaye, S., Hamba, N., Gerbi, A., & Negeri, Z. (2020). Endocrinology & Metabolic Syndrome Oxidative Stress and Carcinogenic Effect of Formaldehyde Exposure: Systematic Review & Analysis. *Endocrinology & Metabolic Syndrome*, *9*(6). DOI: https://doi.org/10.35248/2161-1017.20.9.319

Vaishali Aggarwal, Tuli, H. S., Varol, A., Thakral, F., Yerer, M. B., Sak, K., Varol, M., Jain, A., Khan, Md. A., & Sethi, G. (2019). Role of Reactive Oxygen Species in Cancer Progression: Molecular Mechanisms and Recent Advancements. *Biomolecules*, *4*(1), 88–100.

Wangikar, P. B., Dwivedi, P., Sinha, N., Sharma, A. K., & Telang, A. G. (2005). Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B1 with special reference to microscopic effects. *Toxicology*, *215*(1–2), 37–47. DOI: https://doi.org/10.1016/j.tox.2005.06.022

Wedayani, A. A. A. N., Punagi, A. Q., Pieter, N. A., Cangara, M. H., Kadriyan, H., Susilawati, N. K., Nurhidayati, Putri, N. A., Hidayat, L. H., Makbul, I. H., Fauzan, M., Abdiman, I. M. T., Wiguna, I. M. D., & Kusuma, I. A. J. (2023). *Kandang Tikus Ekonomis Sebagai Media Induksi Displasia UntukStudi Kesehatan* (Patent No. 000473934). URL: www.tcpdf.org

White, M. C., Holman, D. M., Boehm, J. E., Peipins, L. A., Grossman, M., & Henley, S. J. (2015). Age and Cancer Risk. *American Journal of Preventive Medicine*, *46*.

World Health Organization. (2000). *Hazardous Chemicals in Human and Environmental Health*.

World Health Organization. (2022, February 3). *Cancer*. World Health Organization. URL: https://www.who.int/news-room/fact-sheets/detail/cancer

Young, B., O'Dowd, G., & Woodford, P. (2017). Wheater's Functional Histology A Text and Colour Atlas. In *Neuroprosthetics: Theory and Practice: Second Edition*. DOI: https://doi.org/10.1142/9789813207158_0004

Zhang, V. X., Sze, K. M. F., Chan, L. K., Ho, D. W. H., Tsui, Y. M., Chiu, Y. T., Lee, E., Husain, A., Huang, H., Tian, L., Wong, C. C. L., & Ng, I. O. L. (2021). Antioxidant supplements promote tumor formation and growth and confer drug resistance in hepatocellular carcinoma by reducing intracellular ROS and induction of TMBIM1. *Cell and Bioscience*, *11*(1). DOI: https://doi.org/10.1186/s13578-021-00731-0