Journal of Experimental Biology and Agricultural Sciences, December - 2022; Volume – 10(6) page 1486 – 1491

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Gastroprotective activity of yogurt fortified with purple roselle extract in rats exposed with 2,3,7,8-TCDD

Ani Setianingrum*, Syafira Firdhiani, Ajeng Erika Prihastuti Haskito, Ahmad Fauzi

Faculty of Veterinary Medicine, Universitas Brawijaya, Puncak Dieng Eksklusif, Kalisongo, Malang, Indonesia 65151

Received – March 31, 2022; Revision – September 05, 2022; Accepted – December 10, 2022
Available Online – December 31, 2022

DOI: http://dx.doi.org/10.18006/2022.10(6).1486.1491

KEYWORDS

Dioxins

Gastric

MDA

Yogurt

Purple roselle

ABSTRACT

Persistent organic pollutant 2,3,7,8-Tetrachlorodiobenzo-p-dioxin (TCDD), known as harmful congeners of dioxin, has many adverse effects on human or animal health. This TCDD enters the body through inhalation, ingestion, and skin contact. Purple Roselle is a well-known herb-medicinal plant having antioxidant properties. This study aimed to evaluate the antioxidant capacity of purple roselle water extract along yogurt against dioxin exposure. The antioxidant properties of the extract were measured by malondialdehyde (MDA) levels and gastrointestinal histology. For this, 25 white male rats (*Rattus norvegicus*) were used, and these rats were divided into five groups negative control, positive control (TCDD 200 ng/kg BW), and three treatment groups (TCDD 200 ng/kg BW + yogurt fortified with purple roselle water extract concentrations 0.5, 1, and 1.5 percent), all the treatments were given orally. Gastric MDA was determined quantitatively using the Thiobarbituric Acid (TBA) method and the one-way ANOVA test, continued by a Tukey post hoc test with a confidence level of 95% while the gastric histology was observed descriptively. Results of the study revealed that supplementation of fortified yogurt with 1% purple roselle extract could dramatically reduce MDA levels ($p<0,05$) and heal histological damage in the lamina propria mucosa of stomach rats subjected to TCDD. Results of the study can be concluded that consuming yogurt with purple roselle extract can reduce MDA levels and repair histological damage to the gastric mucosa caused by dioxin exposure.

* Corresponding author

E-mail: ani.setia@ub.ac.id (Ani Setianingrum)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

The rapidly growing population, industrial developments, and domestic production activities increased the pollution of soil, water, and air (Fatmawati et al., 2020). Dioxin or dioxin-like compounds are a group of toxic substances that are persistent organic pollutants (POPs) in the environment persists. These compounds are generated by the combustion of compounds containing chlorine and carbon elements. World Health Organization (WHO) reported one of the most harmful dioxin congeners is TCDD (Schecter et al. 2019).

TCDD is the most dangerous pollutant and more than 90% of TCDD exposure to humans is through food, including fish, meat, milk, eggs, and other processed goods (Fatmawati et al. 2020). TCDD is very stable and lipophilic and has low metabolism and excretion, and it is accumulating in the body. Exposure to TCDD can cause adverse health effects, including skin damage, endocrine disorders, reproductive disorders, neurotoxicity, and disruption of biochemical processes in the body due to oxidative stress (Schecter et al., 2019). Consumption of antioxidants is one way to prevent oxidative stress due to exposure to harmful chemicals.

Yogurt has very high nutritional content and health benefits and because of this, it is a well-consumed food throughout the world. Lactic acid bacteria of yogurt release active peptides from precursor proteins during milk fermentation, acting as a scavenger and preventing the generation of free radicals (Mohanty et al. 2016). Fortification of Yogurt with natural antioxidant ingredients from plants increased the antioxidant activity of yogurt. Roselle (*Hibiscus sabdariffa* L) is a natural antioxidant having anthocyanins, vitamin C, flavonoids, and phenolic acids (Izquierdo-Vega et al., 2020). Roselle extract can be used for fortifying yogurt and can be improved the quality of yogurt as a therapeutic nutritional ingredient. The purpose of this study was to evaluate the effect of purple roselle-fortified yogurt in improving the damage of digestive tissue caused by TCDD exposure in white male rats (*Rattus norvegicus*) by observing the gastric MDA levels and gastric histopathology.

2 Materials & Methods

2.1 Materials

The materials used in this study are fresh milk (obtained from local dairy farms in Batu, Malang, Indonesia), Yogurt starter Yógourmet (LYO-SAN. INC 500 Aéropare, C.P. 598, Lachute, QC. Canada, J8H 4G4 containing the bacteria *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus*), Dioxin compound of 2,3,7,8-TCDD with >99% purity (obtained from Supelco, Cat No: 48599), dehydrated flower petals of purple roselle, corn oil, formaldehyde, ethanol, hematoxylin, and eosin dye, and paraffin.

2.2 Purple Roselle Extract Preparation

The aqueous extract of purple roselle was prepared as per the method given by Suharto et al. (2016). For this, 20 g of roselle powder was mixed with 100 ml of water and this mixture was pasteurized at a temperature of 63-65^{o}C for 30 minutes. Pulverized purple roselle was filtered through a 60-mesh strainer. After 30 minutes, the liquid and the sediment were separated.

2.3 Yogurt Making

The procedure for making a liquid starter (mother culture) was based on Padaga et al. (2015). For this, 100 ml of fresh milk was pasteurized in a 250 ml Erlenmeyer tube at 72^{o}C for 15 minutes. This was followed by the cooling of milk (45^{o}C) and mixing of 0.5 g of starter. The prepared mixture was incubated at 45^{o}C for approximately 4 hours to form a yogurt starter. The procedure for making yogurt from a yogurt starter is as proposed by Padaga et al. (2015).

2.4 Making Purple Roselle Extract Fortified Yogurt

The procedure of yogurt fortification by purple roselle extract was as per Suharto et al. (2016). A total of 100 ml of yogurt was homogenized using a blender and the purple roselle extract was added in a predefined concentration of 0.5%, 1%, and 1.5%. After that, it was homogenized again using a blender. Furthermore, fortified yogurt with purple roselle extract was stored in the refrigerator until used.

2.5 Animals Experimental

The twenty-five white male rats (*Rattus norvegicus*) having about 150-200 grams weight and 6-8 weeks of age were obtained from the integrated research and testing laboratory (LPPT) UGM, Yogyakarta, Indonesia. These experimental animals were acclimatized at 25-26^{o}C temperature and 12-hour dark-light cycle under laboratory conditions for seven days. During the period rats were provided with standard commercial feed and drinking ad-libitum. The institutional ethics committee has approved the study under document number 1123.KEP-UB.

Acclimatized twenty-five male white rats were divided into five groups and each group consists of 5 individuals. The formulated five groups are (A) negative control group (given drinking water with a gastric probe), (B) positive control group (experimental animals were exposed to 200 ng/kg BW TCDD only), (C) treatment group 1 (animals exposed with 200 ng/kg BW TCDD and treated with 0.5% purple roselle extract fortified yogurt), (D) treatment group 2 (animals exposed with 200 ng/kg BW TCDD

and treated with 1.0% purple roselle extract fortified yogurt) and (E) treatment group 3 (animals exposed with 200 ng/kg BW TCDD and treated with 1.5% purple roselle extract fortified yogurt). For dioxin exposure, TCDD was dissolved in corn oil and orally given to the experimental rats. The dose of TCDD was determined according to Xu et al. (2008). The treatment group (C, D, E) was given fortified yogurt with purple roselle extract 2 hours after TCDD exposure. Rats were given 1 ml purple roselle extract fortified yogurt at each concentration (0.5%; 1.0%; 1.5%) using a gastric probe orally every day for 12 days. On the day 13th, euthanasia was performed for gastric organ harvesting. For histopathological observation, the gastric organs were put into a 10% formaldehyde solution, and for the examination of MDA levels, the gastric organs were stored at -80 ^{o}C.

2.6 Gastric MDA Level Assay

Gastric malondialdehyde (MDA) levels analyses were carried out by using the thiobarbituric acid (TBA) method. The gastric organs were cut into small pieces of up to 0.1 g, crushed, and added 0.9% physiological NaCl. The homogenate mixture was conveyed to a microtube tube and centrifuged at 1000 rpm for 10 minutes. The supernatant was used for measuring the absorbance at 532 nm with a spectrophotometer and plotted on a standard curve to calculate the sample concentration.

2.7 Gastric Histopathology

Gastric histopathological samples were selected for the pylorus sections and were sequentially prepared by fixation, dehydration, infiltration, embedding, cutting, sticking to object glass, and HE staining. Collected organs were preserved in a 10% formaldehyde solution. This was followed by the dehydration process by immersing the organs in an ethanol solution with a graded concentration for a 1-hour duration for each. Then the clearing was carried out with xylol (I, II) for a 30-60 minutes time period for each. After this, the infiltration process with xylol paraffin (I, II) was carried out for 30 minutes each at 54-56^{o}C. Prepared organs were embedded in paraffin wax and cooled at room temperature. After that, the paraffin block was sliced to a thickness of 3 m, laid on an object glass, dripped with Canada balsam, and finally overlaid with a cover glass. The object glass was then placed in an incubator for one night at 37 ^{o}C. The samples were then stained with hematoxylin and eosin (HE).

Gastric histopathological observations were taken by using Olympus BX 51 light microscope at 40x and 100x magnification. The images were taken using an Olympus XC10 camera. Gastric mucosal erosions and inflammatory cell infiltration were observed in histopathological observations.

2.8 Data analysis

Gastric MDA levels between different groups were determined statistically using one-way ANOVA with a 95% confidence level continued by Tukey's post hoc test. Gastric histopathology was interpreted descriptively.

3 Results

3.1 Gastric malondialdehyde (MDA) levels

Gastric MDA levels of different groups are presented in table 1. Analyzing MDA levels using the TBA method revealed that exposure to TCDD caused an indicative increment ($p<0.05$) in the positive control group (B) and this effect can be reversed by the treatment of natural antioxidants like fortified yogurt with purple roselle extract.

3.2 Gastric Histopathology

Histopathological examination of all groups' stomach pyloric parts is carried out (Figure 1). Based on observations on the gastric histopathology, rats in Group B which were administered TCDD at a dose of 200ng/kg BW for 12 days, show impairments in the mucosal layer, which is characterized by the erosion of the gastric mucosa and infiltration of inflammatory cells. This effect was fewer in the various treatment groups. Further, the slide prepared from treatment group 1 (c) have shown a reduction in mucosa erosion while in the slide of treatment group 2 (D) simplex columnar epithelial mucosa appeared and it did not show any

Table 1 Effect of yogurt roselle combination of the MDA level of TCDD exposed Rat's gastric

Groups	MDA level (ng/mL)
Negative control (A)	207.75±25.85^{b}
Positive control (B)	250.00±6.82^{c}
Treatment 1 (C)	231.05±10.07^{bc}
Treatment 2 (D)	163.00±35.35^{a}
Treatment 3 (E)	239.55±13.27^{bc}

Data are mean of five replicates; ± Standard Error of mean; Values without common letters differ significantly at LSD $P<0.05$; Positive control (TCDD exposed rats), Treatment 1 (TCDD + yogurt roselle 0.5%), Treatment 2 (TCDD + yogurt roselle 1%), Treatment 3 (TCDD + yogurt roselle 1.5%)

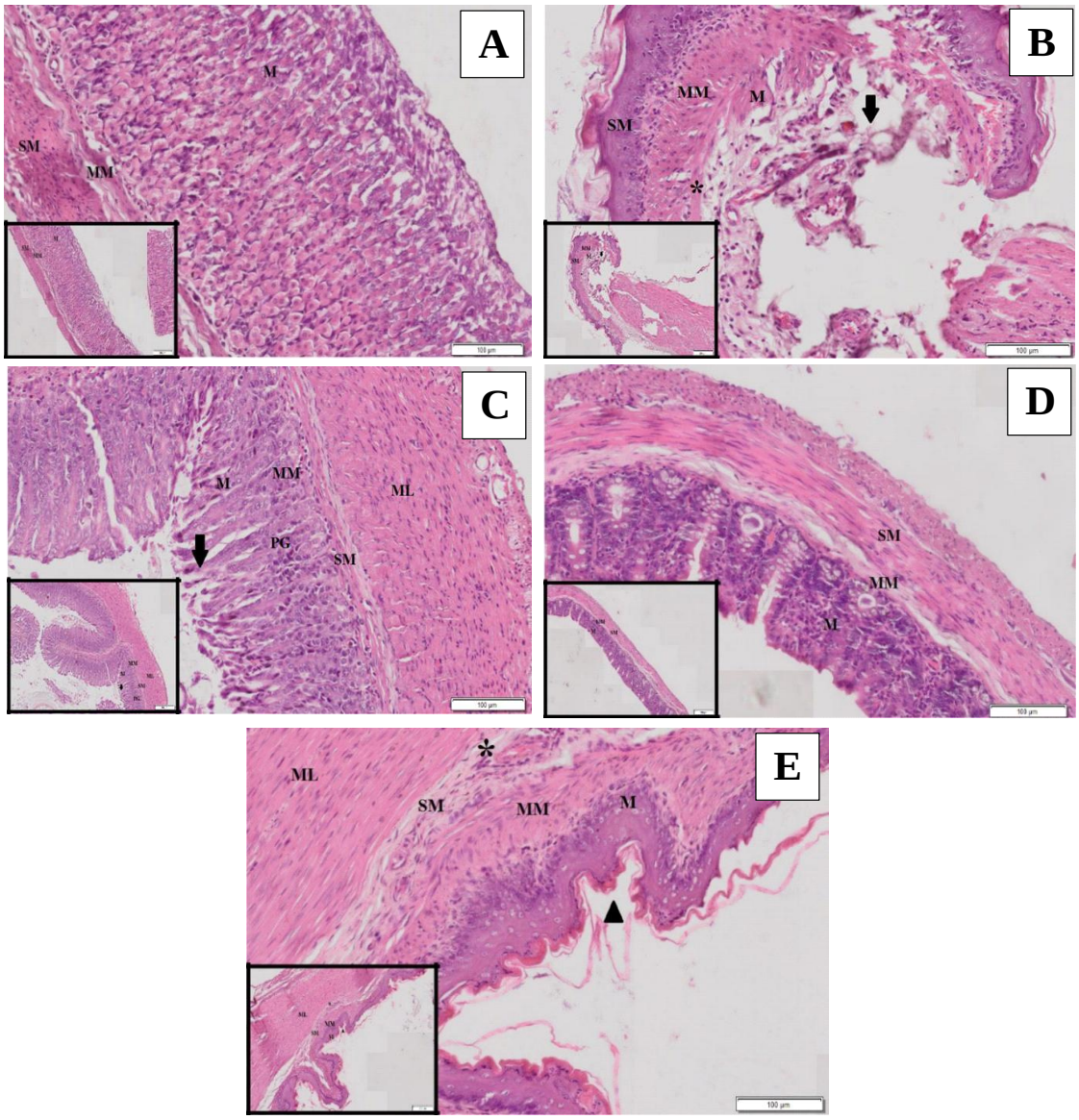

Figure 1 Gastric histopathology of the pylorus of Rat (*Rattus norvegicus*): negative control (A), positive control (B), Treatment 1 (C), Treatment 2 (D), and Treatment 3 (E). HE staining; magnification 400x (inset:100x).M (Mucosa); MM (Muskularis Mucosa); SM (Submucosa)); ML (*Muscle Layer);*PG (*Pyloric Glands*); *(inflamation cell infiltration); ↓(gastric mucosa erotsion); ▲(mucosal desquamation)

gastric mucosal erosion and it delivers results close to the stomach of group A. In the case of group 3 (E), the epithelial mucosa was not intact and showed epithelial desquamation and slight inflammatory cell infiltration.

4 Discussion and Conclusions

Administration of 200 ng/kg BW TCDD for 12 days can cause damage to gastric cells. Exposure to TCDD can cause oxidative stress that started from the entry of TCDD into the body. First of all, TCDD penetrates the cell membrane, binds to Aryl hydrocarbon receptor (AhR) located in the cytosol, and then moves to the cell nucleus. Here in the cell nucleus, the Ah receptor binds with TCDD to form a dimer with Aryl hydrocarbon receptor nuclear translocator (ARNT) protein. Then, the complex AhR-TCDD-ARNT bind with the Dioxin Response Element (DRE), and this binding will increase the expression of cytochrome P450, especially CYP1A1 (Vijaya et al. 2014). Cytochrome P450 was involved in the chain-forming free radicals in the body and the accumulation of a large number of free radicals in the body can trigger oxidative stress. Further, oxidative stress has an impact on damaging several critical cellular components such as fats, proteins, and DNA. Damage due to exposure to free radicals in fats triggers the cell membranes' lipid peroxidation with MDA

metabolite products (Mahdi et al. 2019). Further, induction of TCDD increases the concentration of COX-2 cyclooxygenase enzyme, which affects the synthesis of prostaglandins and increases the inflammatory process in tissues (Mahmoud 2020). Exposure to TCDD also causes impairment in the histopathological appearance of the gastric mucosa. According to Mahmoud (2020), TCDD also damaged the fundus of the stomach in various ways, including irregular fundic glands, desquamation epithelium, inflammatory cells, and dilatation of blood vessels. In addition, hyperplasia and changes in the gastric mucosa, and hyperplasia of enteroendocrine cells were also reported after exposure to TCDD. Similarly, Amer et al. (2013) reported that the toxic effects of TCDD cause glandular degeneration, apoptosis, and gastric ulcers.

Various treatment groups (C-E) have shown recovery from the hepatotoxicity caused by TCDD. Exposure to toxic substances may cause reverse diffusion of H+ from the lumen to the mucosa in the stomach. This mechanism causes mucosal damage followed by pepsin released in large quantities for Na+ ions, and plasma proteins enter the lumen. After that, the body will release histamine and increases gastric acid and capillary permeability. The muscular mucosal tonus will increase, and mucosal erosion occurs.

Based on histopathological observations of the treatment groups 1, 2, and 3 gastric, it was reported that histopathological damage can be prevented by giving yogurt fortified with purple roselle extract. Lactic acid bacteria in yogurt have activity as anti-allergic, anti-inflammatory, anti-cancer, and gastroprotective activities (Gomi et al. 2013). Proteolytic enzymes from LAB can enhance the release of active peptides from precursor proteins in milk. According to Mohanty et al. (2016), biopeptides present in yogurt function as antioxidants and help in free radical scavenging. Further, Rodríguez et al. (2009) also suggested that the content of lactalbumin in dairy products acts as a gastroprotective, and stimulates mucin synthesis and secretion of mucus-producing cells to protect the stomach from damage. In addition, anthocyanins present in purple roselle also have antioxidant effects, and free radical scavenging ability (Khoo et al. 2017).

The daily dose of yogurt-fortified purple roselle extract showed a gastroprotective effect on gastric MDA levels in TCDD-exposed rats and this effect was reported highest in treatment group 2. According to Mohanty et al. (2016), bioactive peptides present in yogurt reduce MDA levels by inhibiting lipid peroxidation and free radical-scavenging activity. In addition, anthocyanins contained in purple roselle can also prevent oxidative stress by stabilizing unpaired free radical electrons through hydrogen atom donors (H) (Apáez-Barrios et al. 2018).

Yogurt fortified with purple roselle extract treatment showed a gastroprotective effect against the toxic effects of TCDD exposure. The synergistic antioxidant activity of yogurt and purple roselle extract can protect the gastric mucosa from damage caused by cell oxidation. In this study, the concentration of fortified purple roselle extracts 1% in yogurt best prevented gastric histopathological damage in rats (Rattus norvegicus) exposed to TCDD. According to the findings, yogurt supplemented with purple roselle extract can reduce stomach MDA levels and gastric histological damage caused by TCDD.

Acknowledgment

Universitas Brawijaya provided research funding for this work. The authors show gratitude to LPPM UB, Biosains Institute UB, and the Faculty of Veterinary Medicine Universitas Brawijaya.

Conflict of interest and financial disclosures

No relevant conflicts of interest for the authors to disclose in this article.

References

Amer, M. G., Mohamed, D. A., & Karam, R. A. (2013). Protective role of curcumin against 2,3,7,8-tetrachlorodibenzodioxin- induced histological and biochemical changes in fundic mucosa of the adult rat gastric. *Egyptian Journal of Histology*, *36*(1), 13–27.

Apáez-Barrios, P., Pedraza-Santos, M. E., De Las Nieves Rodríguez-Mendoza, M., Raya-Montaño, Y. A., & Jaén-Contreras, D. (2018). Performance and concentration of anthocyanins in *Hibiscus sabdariffa* L. with 5 foliar application of micronutrients. *Revista Chapingo, Serie Horticultura, XXIV* (*2*), *2018*. DOI: 10.5154/r.rchsh.2017.06.020

Fatmawati, M., Nugroho, W., Setianingrum, A., & Haskito, A. E. P. (2020). *Kesehatan Masyarakat Veteriner: Kesehatan Susu, Telur, Daging, dan Lingkungan*. Universitas Brawijaya Press.

Gomi, A., Harima-Mizusawa, N., Shibahara-Sone, H., Kano, M., Miyazaki, K., & Ishikawa, F. (2013). Effect of Bifidobacterium bifidum BF-1 on gastric protection and mucin production in an acute gastric injury rat model. *Journal of dairy science*, *96*(2), 832–837. https://doi.org/10.3168/jds.2012-5950

Izquierdo-Vega, J. A., Arteaga-Badillo, D. A., Sánchez-Gutiérrez, M., Morales-González, J. A., et al. (2020). Organic acids from Roselle (Hibiscus sabdariffa L.)-A brief review of its pharmacological effects. *Biomedicines*, *8*(5), 1–16.

Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & nutrition research*, *61*(1), 1361779. https://doi.org/10.1080/16546628.2017.1361779

Mahdi, C., Haskito, A. E. P., Padaga, M. C., & Roosdiana, A. (2019). The activity of casein derived from goat milk yogurt as an antioxidant on histopathology of rat' s liver exposure by 2 , 3 , 7 , 8-tetrachlorodibenzo-p- dioxin (TCDD). *Journal of Physics Conference Series 1146*(1), *012016*. doi:10.1088/1742-6596/1146/1/012016.

Mahmoud, M. (2020). Gastroprotective Activity of Olive Leaves Extract on 2,3,7,8 Tetrachlorodibenzo-p-dioxin Induced Gastric Fundic Mucosal Injury in Adult Male Albino Rats (Light and Electron Microscopic Study). *Egyptian Journal of Histology*. DO - 10.21608/ejh.2020.21248.1215.

Mohanty, D. P., Mohapatra, S., Misra, S., & Sahu, P. . (2016). Milk derived bioactive peptides and their impact on human health – A review. *Saudi Journal of Biological Sciences*, *23*(5), 577–583.

Padaga, C., Aulanni'am, A., Sujuti, H., & Widodo. (2015). Blood Pressure Lowering Effect and Antioxidative Activity of Casein Derived from Goat Milk Yogurt in DOCA-salt Hypertensive Rats. *International Journal of PharmTech Research*, *8*(6), 322–330.

Rodríguez, C., Medici, M., Rodríguez, A. V., Mozzi, F., & de Valdez, G. F. (2009). Prevention of chronic gastritis by fermented milks made with exopolysaccharide-producing Streptococcus thermophilus strains. *Journal of Dairy Science, 92* (6), 2423-2434.

Schecter, A. J., Colacino, J. A., & Birnbaum, L. S. (2019). Dioxins: Health Effects. In J. Nriagu (Ed.), *Encyclopedia of Environmental Health (Second Edition)* (pp. 135–142). Elsevier.

Suharto, E. L. S., Arief, I. I., & Taufik, E. (2016). Quality and Antioxidant Activity of Yogurt Supplemented with Roselle during Cold Storage. *Media Peternakan*, *39*, 82–89.

Vijaya, V., Palaniswamy, P., & Selvi, K. (2014). Protective effect of ellagic acid against TCDD-induced renal oxidative stress : Modulation of CYP1A1 activity and antioxidant defense mechanisms. *Molecular Biology Reports*, *41*, 4223–4232.

Xu, J. P., Yin, Y. P., & Zhou, X. Q. (2008). Effect of vitamin E on reproductive function in the mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicology and industrial health*, *24*(9), 595–601. https://doi.org/10.1177/0748233708100092