RESEARCH ARTICLE

Effect of *Phylantus ninuri* leaf extract on kidney and liver histopathological features in formaldehyde-exposed rats

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ABSTRACT

Formaldehyde (FA) metabolism in the body reduces glutathione levels, resulting in liver and kidney damage. *Phylantus ninuri (meniran)* has been demontrated to have hepatoprotective properties. However, the effect of *meniran* administration on the histological features of the liver and kidney in in formaldehyde-exposed rats has not been adequately studied. The study was aimed to determine the effect of *meniran* on the histopathological feature of liver and kidney cell. This was a posttest randomized control group design. A total of 24 male Wistar rats were divided into 4 groups of 6 rats each. Group 1 was a control group that received only oral formaldehyde. Group 2, Group 3, Group 4 was administered oral formaldehyde together with *meniran* extract at dose of 100 mg/kg BW, 200 mg/kg BW, and 300 mg /kg BW respectively for 14 days. The histopathological changes of the kidneys and liver were assesed using hematoxylin and eosin (HE) staining. The mean of kidney and liver damage in the *meniran* group at various doses was lower than that of control group. There was a significant difference in the mean of kidney damage between the control group and the *meniran* group at various doses (p<0.05). The mean histopathological score of liver damage showed a significant difference between the control group and various treatments (p<0.05). Ethanolic extract of *meniran* leaves ameliorates liver and kidney damage in formaldehyde-exposed rats.

1. Introduction

Formaldehyde (FA) is toxic agent commonly used as preservative. The use of formaldehyde to preserve corpses or tissues for educational purposes has been well studied. As toxic agent, formaldehyde can be detected as contaminants in foods like grains, fruits due to fumigation. In addition, it can be found in food that has been heated in tableware containing formaldehyde resin. Formaldehyde has been employed as a bacteriostatic agent in the production of a variety of foods, including cheese (WHO, 2020). Under normal conditions, the human body produces approximately 100 M of endogenous formaldehyde, a quantity that increases with demethylation/deamination and myeloperoxidase enzymatic reactions. Endogenous formaldehyde is also an intermediate compound in the biosynthesis of purines and certain amino acids, including lysine, L-methionine, histamine, and methyl alanine (Bernardini *et al.*, 2020; Nakamura *et al*, 2017).

Oral administration of formaldehyde can cause damage to gastrointestinal tract, especially in the gastric and esophageal tissues. The toxicity of the formaldehyde is due to its high-water solubility and high reactivity with neutrophilic groups found in protein, DNA and RNA molecules (Bernardini *et al.*, 2020). Research conducted by Ke *et al.* (2014) showed that formaldehyde

at dose of 125 µM causes cell death and increases intracellular ROS levels. Kim *et al*. (2018) showed that administration of formaldehyde causes epithelial cell death and elevated inflammatory response markers indicated by elevated TNF levels. Increased in cell death occur through associated apoptotic mechanisms with decreased expression of matrix metalloprotein (MMP) and increased activity of cytochrome oxidase, caspase 3/7 and caspase 9. Manurung *et al*. (2017) showed that oral administration of formaldehyde at the dose of 100 mg/kg BW for 14 days cause kidney damage in rat especially in the renal corpuscle and proximal tubule compared to control group. Rindwitia *et al*. (2015) demonstrated increased SGOT and SGPT levels in Wistar rat after oral formaldehyde administration at the dose of 0.1 mL/kg BW orally for 14 days. Several studies demonstrated that toxic effect of formaldehyde to kidney and liver is associated with increased free radicals and inflammation. Thus, the administration of antioxidant and anti-inflammation has been proposed to repair of kidney and liver damage.

Phylantus ninuri (*meniran*) used in folk medicine as immunomodulator has been shown to have a potential to increase the level of endogenous antioxidants such as glutathione. Sutrisna *et al*. (2019) revealed that ethanolic leaf extract of *meniran* contains alkaloids, triterpenoids, saponins, tannins and flavonoids. *Meniran* at a dose of 250 mg/kgBW has been shown to have anti-inflammatory effect indicated by the significant decrease in area under curve (AUC) compared to control negative. Flavonoid in the form of rutin, gallic acid and quercetin which has been reported to be a potent antioxidant activity in vitro (Rusmana *et al*., 2017). l35KDa protein isolated from *meniran* has been shown to have a potent antioxidant activity and cytoprotective antioxidant activity on liver cells. In general, flavonoids isolated from a number of plants have powerful antioxidant effect (Panche *et al*., 2016).

The administration of *meniran* extract has been shown to have a protective activity against formaldehyde-induced kidney and liver damage. However, there have been limited studies on the effect of *meniran* ethanolic extract on histopathological features of liver and kidney. This study aimed to evaluate the effect of the administration of *meniran* on formaldehyde induced kidney and liver damage in rats based on the histopathological features.

2.   Materials and Methods
2.1.   Research design

This was a posttest randomized control group design study conducted in the Integrated Biomedical Laboratory (IBL), Faculty of Medicine, Universitas Islam Sultan Agung. The ethical clearance was obtained from the ethics committee of the Faculty of Medicine, Universitas Islam Sultan Agung under ethic number of 428/XII/2021.

2.2.   Extract Preparation

Meniran was obtained from the traditional market in Semarang, Central Java, Indonesia. 250 g of *meniran* leaves were dried with the oven and powdered using a grinder to obtain 235 g dry powder. *Meniran* was extracted using maceration by drenching 235 g of it in 1 L of analytic ethanol for 24 hours. The mixture was filtered and concentrated on rotary evaporator at 400 C to obtain 17.4 g concentrate.

2.3.   Treatment administration

A total of 24 Wistar rats weighing 150–200 g was adapted for 7 days before 14-day treatment. During adaptation and treatment, the rats were fed with standard diet *ad libitum.* The rats were divided into four groups of six rats each. All groups received 0.2 cc oral formaldehyde at the dose of 100 mg/kg BW. control group (Group I) received only formaldehyde. The treated groups were administrated with *meniran* at dose of 100 mg/kg BW (group II), 200 mg/kg BW (group III), and 300 mg/kg BW (group IV). *Meniran* at various dose was administrated 2 hours after the administration oral formaldehyde. On day15, all rats were sacrificed. The kidney and liver samples were taken and fixed in formaldehyde buffer solution for 7 days then prepared for histopathology staining using hematoxylin eosin dye.

2.4.   Determination of histopathological feature of liver and kidney cell

The observation was conducted under microscope at 400x magnification at 5 field of view. Reading of the preparations to determine categories and calculations of the number of cell damage done by expert of pathology anatomy without replicates.

In the present study, cell damage of kidney and liver tissue was classified as albuminous degeneration, hydropic degeneration, fat degeneration and necrotic cells. On histopathological assay, both kidneys and liver hydropic degeneration was observed by visible clear cavities in cytoplasm. Hydropic degeneration is reversible due to disturbance of Na/K^+ pump leading to the entrance of fluid into cells. Albuminous degeneration was indicated by paler cytoplasm compared to normal cells. Degeneration albuminous on preparations is marked with swelling cytoplasm, while dead cells was indicated with solid, pycnotic cells.

Mean of damaged cells were determined using the modified previously proposed methods (Almunawati *et al*., 2017; Klopfleisch, 2013; Susianti,

2013) as follows:

$$\frac{(D=(a \times 2)+(b \times 2)+(c \times 3)+(d \times 4)}{5}$$

where D is the mean of damaged cells, a is the number of albuminous degenerations, b is the number of hydropic degenerations, c is the number of fat degenerations, d is the number of necrotic cells.

2.5. Data Analysis

Data obtained were presented in form mean ± SD and analyzed using Kruskal Wallis test using SPSS 22.0.

3. Results
3.1. Histopathological feature of kidney cells

The study showed that formaldehyde administration can increase histologic score of kidney damage. The cell damage found in the formaldehyde-exposed rats (Group I) was albuminous degeneration, fat degeneration and necrotic cells. The cell damage in the kidney in Group II included albuminous, hydropic, and fat degeneration, not to mention cell death in the form of necrosis or apoptosis. Group III shows damage of kidney tissue in the form of hydropic degeneration and cell death, while group IV showed albuminous and fat degeneration (figure 1). Kidney cell degeneration was observed in the area proximal tubule. Histopathological

features of renal proximal tubule were presented by vacuole degeneration in the cytoplasm, while hydropic degeneration is marked with clear vacuole due to fluid accumulation in the cytoplasm, and cell death was characterized with a small nucleus (Figure 1).

The mean of cell damage number (D) in the kidneys of group II was found to be higher compared with group I. The lowest score was found in group IV (rats exposed to formaldehyde and treated with *meniran* at dose of 200 mg/kg BW) (Table 1). The kidney cell damage in group I showed no significant different compared with groups III and IV (p > 0.05). Mean kidney cell damage in group II was significantly different compared with groups I, III and IV (p<0.05). D value in group III showed no significant difference compared with group IV (0.078).

Table 1. The mean of cell damage number (D) of kidney in the various groups

Group	Mean ± SD	Sig.
I	64.83 ± 32.31	0.008*
II	177.83 ± 23.42	
III	41.00 ±8.17	
IV	19.67 ± 7.93	

(*) Kruskal Wallis results shows a difference among groups at 5% significance level
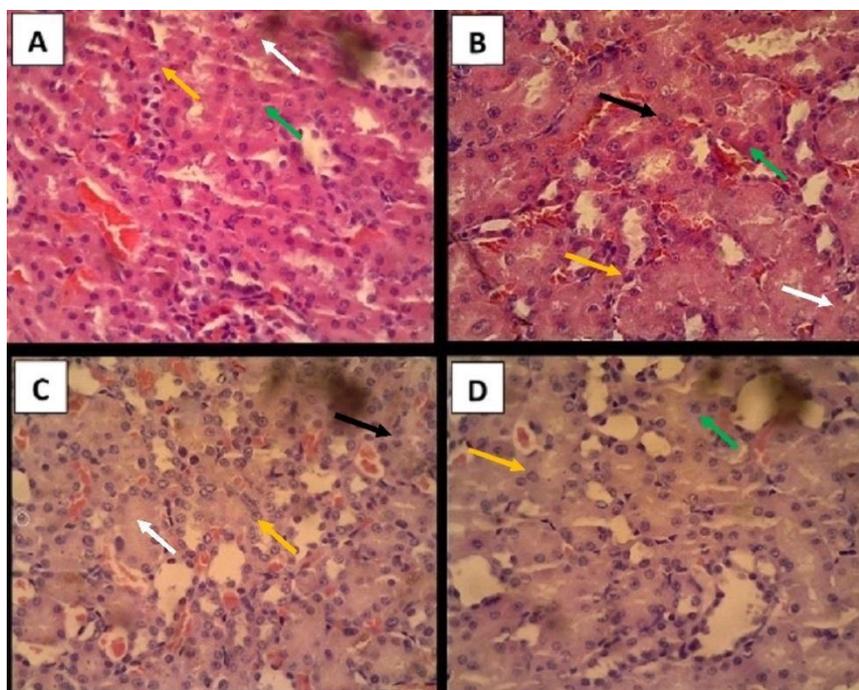
Figure 1. Histopathological features of kidney in formaldehyde-exposed rats at different treatment groups: (A) group I (formaldehyde only); (B) group II (formaldehyde and 100 mg/ kg BW *meniran*); (C) Group III (formaldehyde and 200 mg/ kg BW *meniran*); (D) Group IV (formaldehyde and 300 mg/ kg BW *meniran*). Yellow arrow shows albuminous degeneration, black arrow shows hydropic degeneration, green arrow shows fat degeneration, and white arrow shows necrotic /apoptotic cells.
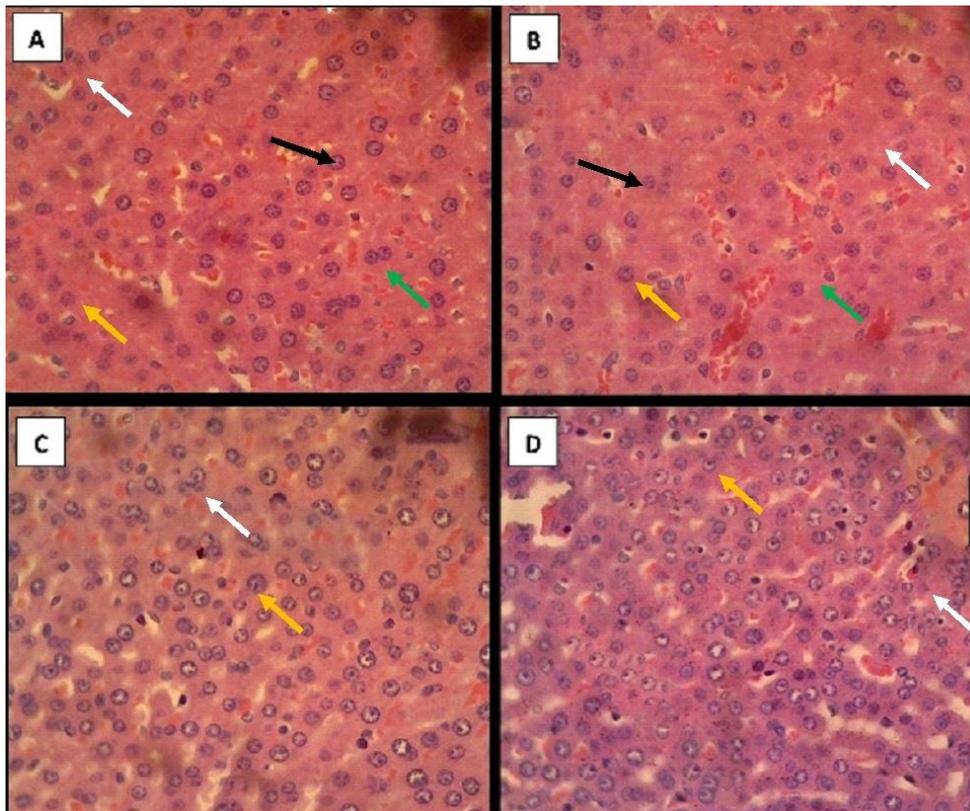
Figure 2.   Histopathological features of liver in formaldehyde-exposed rats at different treatment groups: (A) group I (formaldehyde only); (B) group II (formaldehyde and 100 mg/ kg BW *meniran*); (C) Group III (formaldehyde and 200 mg/ kg BW *meniran*); (D) Group IV (formaldehyde and 300 mg/ kg BW *meniran*). Yellow arrow shows albuminous degeneration, black arrow shows hydropic degeneration, green arrow shows fat degeneration, and white arrow shows necrotic/ apoptotic cells.

Table 2.   The mean of cell damage number (D) in liver of rats exposed to formaldehyde of different groups

Group	mean ± SD	Sig.
I	204.17 ± 54.72	0.000*
II	113.83 ± 41.32	
III	40.83 ± 25.44	
IV	13.67±7.56	

(*) a difference among groups based on Kruskal Wallis test

3.2.   Histopathological feature of liver cells

Histological features of liver in groups I and II showed a hydropic degeneration and albuminous degeneration, fat degeneration and cell death in the form of necrosis or apoptosis. Groups III and IV showed liver damage in the form of degeneration albuminous and dead cell (Figure 2). The mean of cell damage number (D) in the liver was presented at Table 2.

The mean of cell damage number (D) in liver of group I was significantly different compared to groups II, III and IV ($p <0.05$). Mean histologic score of cell damage in the liver in group II was significantly different compared with groups III and IV ($p < 0.05$). Mean histologic score in group III was significantly different from group IV ($p < 0.05$).

4.   Disscussion

The present study showed that formaldehyde administration can damage the liver and kidneys in the proximal tubule indicated by the reversible degeneration and irreversible necrosis. The finding is in line with that of previous study done by Manurung, *et al*. (2017). Ingestion of formaldehyde, which enters the bloodstream and is subsequently converted to formic acid, causes irreversible damage by interfering with electron transport and ATP formation in the mitochondria, resulting in cell death (Zararsiz *et al*., 2007). On the other hand, reversible damage begins with oxidative stress oxidative caused by formaldehyde exposure, that will trigger the generation of reactive oxygen species (ROS) in the kidney as well as in the liver.

The increase in ROS is due to the decrease of glutathione that is used as cofactor by formaldehyde dehydrogenase enzyme in the process of formaldehyde metabolism to form formic acid (Golalipour *et al*., 2009). Manurung *et al.* (2017) reported that hydropic

degeneration is due to Na$^+$/K ATPase pump failure in maintaining ionic and fluid homeostasis (Manurung *et al.*, 2017). Research results showed that most of the cell damage are reversible, so that the administration of antioxidant-rich *meniran* in has been suggested to repair it. Mahdi's research (2010) proved that that formaldehyde administration caused a change in protein profile in the form of increased the protein expression of 29.6 KDa in the suspected liver in the form of heat shock proteins. Wibowo (2012) reported that oral administration of formaldehyde at various doses for 12 weeks cause changes in the histopathological feature of kidney with increase degeneration, atrophy, and tubules dilatation.

Formaldehyde inside body is oxidized into formic acid by formaldehyde dehydrogenase (FDH) enzyme. The process needs glutathione as FDH cofactor; thus, metabolism of formaldehyde is associated with the decrease in the glutathione level leading to the increased toxicity of formaldehyde (Zarsiz *et al.*, 2007).

Administration of formaldehyde can cause cell death in kidney (Figure 1) and liver (Figure 2) tissues. The elevated levels of TNF- and IL-1 could be responsible for the observed phenomenon. Increased inflammatory response can result in cell death via apoptosis and necrosis. The finding is in line with previous study showing that formaldehyde exposure can increase inflammation and DNA damage leading to cell death. The cell death due to formaldehyde exposure include necrosis and apoptosis. Mechanism of apoptosis due to formaldehyde exposure occurs through intrinsic pathway which involve increased caspase-3 due to increased free radicals (Bernardini *et al.*, 2020).

In the present study, the administration of *meniran* ethanolic extract at various doses showed a significant difference in the histologic score of cell damage showed by the varied of the mean of cell damage number (D). That might have been associated with effect antioxidant and anti-inflammatory from active substance of *meniran* such as flavonoids, tannins, phenolics, and terpenoids (Geethangil & Ding, 2018). The finding is also in line with an in-vitro study reporting the antioxidant activity of *meniran* on doxorubicin-induced cardiotoxicity experiment (Chularojmontri *et al.*, 2005).

A study performed by Tumnbuan *et al.*, (2019) found that herbal extract of *meniran* fulfill the requirements of the quality standard. Phytochemical screening of herbal powder of *meniran* showed flavonoid, saponins, tannins, quinones, triterpenoids, coumarins and essential oils. The IC$_{50}$ value of 70% ethanolic extract of *meniran* is: 17.55 bpj, 17.64 bpj, 17.59 bpj is classified into strong antioxidants due to the interaction among secondary metabolites.

Ethanolic extract of *meniran* at the dose of 200 and 300 mg/ kg BW decreased the mean of damaged cells number in the liver and kidneys. That might be associated with its antioxidants properties. However, the administration of *meniran* at the dose of 100 mg/ kg body, cause increased number of damaged cells damage cells in tissue kidney. This may be the result of the interaction between the secondary technical implementation of the study's non-invasive laboratory procedure and the uncontrolled environmental conditions of the treatment.

5.   Conclusions

Ethanolic extract of *meniran* decreased the mean of damaged cells number in the liver and kidneys of formaldehyde-exposed rats, thus it can ameliorate liver and kidney damage.

Acknowledgment

References

Almunawati., A, H., Aliza, D. (2017). Histopatologi ginjal tikus putih *(Rattus norvegicus)* yang diinjeksi formalin. *Jurnal Ilmiah Mahasiswa Veteriner,* 1(3), 424–431. https://doi.org/10.21157/jim%20vet..v1i3.3372

Bernardini, L., Barbosa, E., Charão, M.F., Brucker, N. (2020). Formaldehyde toxicity reports from in vitro and in vivo studies: a review and updated data. *Drug and Chemical Toxicology,* 45 (3), 1–13. https://doi.org/10.1080/01480545.2020.1795190

Chularojmontri, L., Wattanapitayakul, S. K., Herunsalee, A., Charuchongkolwongse, S., Niumsakul, S., Srichairat, S. (2005). Antioxidative and cardioprotective effects of *Phyllanthus urinaria* L. on doxorubicin-induced cardiotoxicity. *Biological & Pharmaceutical Bulletin,* 28 (7), 1165–1171. https://doi.org/10.1248/bpb.28.1165

Geethangili, M., Ding, S.T. (2018). A Review of the phytochemistry and pharmacology of *Phyllanthus niruri* L. *Frontiers in Pharmacology*, 9, 1109. https://doi.org/10.3389/fphar.2018.01109

Ke, Y. J., Qin, X. D., Zhang, Y. C., Li, H., Li, R., Yuan, J. L., … Ding, S. M. (2014). In vitro study on cytotoxicity and intracellular formaldehyde concentration changes after exposure to formaldehyde and its derivatives. *Human and Experimental Toxicology*, 33 (8), 822–830. https://doi.org/10.1177/0960327113510538

Kim, S. H., Choi, J. W., Suh, M. W., Lee, J. H., Oh, S. H., Song, J. J., Park, M. K. (2018). Effect of Formaldehyde on human middle ear epithelial eells. *BioMed Research International*, https://doi.org/10.1155/2018/6387983

Klopfleisch, R. (2013). Multiparametric and semiquantitative scoring systems for the evaluation of mouse model histopathology - a systematic review. *BMC Veterinary Research*, 9, 1–15. https://doi.org/10.1186/1746-6148-9-123

Mahdi, C., Aulaniam, A. (2010). The effect of formaldehyde exposure and yogurt supplementation on of *(Rattus norvegicus)*. *Indonesian Journal Chemistry*. 10 (1), 132-137. https://doi.org/10.22146/ijc.21493

Manurung, M.S., Trianto, H.F., Ilmiawan, M.I., Handini, M. (2017). Pengaruh Pajanan Akut Formaldehid per Oral terhadap Gambaran Histologis Korteks Ginjal Tikus Putih Jantan Galur Wistar. *Journal Cerebellum*, 3 (1), 718–730. https://jurnal.untan.ac.id/index.php/jfk/article/view/23580/18506

Nakamura, J., Shimomoto, T., Collins, L. B., Holley, D. W., Zhang, Z., Barbee, J. M., … Bultman, S. J. (2017). Evidence that endogenous formaldehyde produces immunogenic and atherogenic adduct epitopes. *Scientific Reports,* 7 (10787). 1-12. https://doi.org/10.1038/s41598-017-11289-8

Panche, A. N., Diwan, A. D., Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, 5 (c47), 1–15. https://doi.org/10.1017/jns.2016.41

Peanasari, A.R.I., Djamil, S.L., Rohmani, A. (2015). Pengaruh formalin per oral terhadap kadar SGOT dan SGPT tikus Wistar. *Jurnal Kedokteran Muhammadiyah.* 2 (1), 34-38. https://jurnal.unimus.ac.id/index.php/kedokteran/article/view/1751

Rusmana, D., Wahyudianingsih, R., Elisabeth, M., Balqis, B., Maesaroh, M., Widowati, W. (2017). Antioxidant activity of *Phyllanthus niruri* extract, rutin and quercetin. *The Indonesian Biomedical Journal,* 9 (2), 84–90. https://doi.org/10.18585/inabj.v9i2.281

Susanti, S. (2013). Pengaruh ekstrak jintan hitam (*Nigella sativa* L.) terhadap gambaran histopatologi hepar, paru, dan testis tikus putih (*Rattus Norvegicus*) yang diinduksi Gentamisi. *Journal Sainsmat,* II (2), 107–118. https://doi.org/10.35580/sainsmat228282013

Sutrisna, E., Maryati , Wahyuni , S., Tanti Azizah , S. (2019). Anti-inflammatory effect of *Phyllanthus niruri* L. from Indonesia (Pre-clinical Study). *Pharmacognosy Journal*, 11 (6), 1347–1350. https://doi.org/10.5530/pj.2019.11.208

Tambunan, R.M., Swandiny, G.F., Zaidan, S. Uji aktivitas antioksidan dari ekstrak etanol 70% herba *Meniran* (*Phyllanthus niruri* L.) terstandar. *Sainstech Farma Jurnal Ilmu Kefarmasian*. 12(2), 60-64. https://doi.org/10.37277/sfj.v12i2.444

WHO. (2000). Air quality guidelines for Europe, 2nd ed.. World Health Organization. Regional Office for Europe. https://apps.who.int/iris/handle/10665/107335

Wibowo, M., Soeharto, G., Margawati, A. (2012). Pengaruh formalin peroral dosis bertingkat selama 12 minggu terhadap perubahan gambaran histopatologis ginjal tikus Wistar. *Jurnal Media Medika Muda Karya Tulis Ilmiah*. 1-16. https://media.neliti.com/media/publications/116931-ID-pengaruh-formalin-peroral-dosis-bertingk.pdf

Zararsiz, I., Sarsilmaz, M., Tas, U., Kus, I., Meydan, S., Ozan, E. (2007). Protective effect of melatonin against formaldehyde-induced kidney damage in rats. *Toxicology and Industrial Health*, 23(10), 573–579. https://doi.org/10.1177/0748233708089022