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Research Paper

Effect of *Bidens pilosa* L. Leaf Extract on Formalin-Induced Rat Kidney: In vivo and In Silico Study

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ABSTRACT

Background: Formalin, as formaldehyde dissolved in water, is a carcinogen that causes oxidant and antioxidant imbalance. The metabolic products of formaldehyde, such as formic acid, stimulate oxidative stress, leading to kidney damage. *Bidens pilosa* L. (*B. pilosa* L.) leaves contain flavonoids with antioxidant and anti-inflammatory activities. The present study aimed to determine the nephroprotective effect of *B. pilosa* L. leaf extract on the histological structure and weight of the kidney organs of white rats (*Rattus norvegicus*) induced by formalin.

Methods: Rats were grouped into five treatment groups, including normal control, negative control, and three dose groups (25 mg/kg BW, 50 mg/kg BW, and 100 mg/kg BW). Rats were induced with formalin 0.2 ml/kg BW for 7 days orally, followed by dose treatment for 7 days. Kidney histology and weight were examined on day 14. Antioxidant and anti-inflammatory activities were predicted by molecular docking.

Results: The kidney weight in the treatment was not significantly different, but histologically, the *B. pilosa* L. leaf extract could significantly reduce necrosis cells at a dose of 100 mg/kg to approach the score of the normal group. Secondary metabolites, such as isochlorogenic acid and luteolin, had antioxidant and anti-inflammatory properties, which contribute to therapy.

Conclusion: *B. pilosa* L. leaf extract can potentially protect nephrons with antioxidant and anti-inflammatory activities.

Keywords: *Bidens pilosa* L., Formaldehyde, Formalin, Kidney, Rat

Introduction

Formalin consists of 37-40% formaldehyde dissolved in water and 5-12% methanol as a stabilizer [1,2]. Formaldehyde is classified as an A-class carcinogen by the International Agency for Research on Cancer (IARC) [3]. Formaldehyde exposure may induced by inhalation or ingestion. Formalin is produced endogenously by cellular metabolism, such as folate metabolism. Endogenous release of formaldehyde leads to extensive DNA damage, resulting in hepatic and renal dysfunction, cancer, and leukemia [4]. Exogenous exposure to formaldehyde can exacerbate the damage. Exogenous exposure comes from environmental sources, including cigarette smoke, contamination from textiles, plastics, cosmetics, or diet [4]. Although formaldehyde is produced naturally in foodstuffs, such as meat, fish, and vegetables, formaldehyde has been found as an illegal preservative in food [2]. Therefore, the U.S. Environmental Protection Agency set the daily human tolerable consumption limit at 0.2 mg/kg/day, while the World Health Organization (WHO) set the daily limit at 0.15 mg/kg/day [2].

High formaldehyde concentrations induce cytotoxicity, necrosis, and carcinogenic effects that cause inflammatory reactions, protein denaturation, and increased free radicals. Formaldehyde and reactive oxygen species (ROS) are involved in a mutually stimulating cycle with each other [3]. Alcohol dehydrogenase (ADH5) and formate dehydrogenase (FDH) metabolize formaldehyde to a less reactive molecule called formic acid to maintain low intracellular formaldehyde concentrations. Formic acid is oxidized to produce carbon dioxide and water [5] and cannot metabolize completely. The excess formate is excreted in urine and feces [3]. Accumulation of formate in renal tissue leads to metabolic acidosis and acid-alkaline imbalance, which impairs renal function [6].

Bidens pilosa L. (*B. pilosa* L.) is an herbaceous plant of the Asteraceae family. *B. pilosa* L. plants contain 301 active compounds that include polyacetylene, phenolic acids, terpenes, pheophytin, fatty acids, phytosterols, and flavonoids [7]. Flavonoids are characterized by bioactivity properties, including anticancer, antioxidant, and anti-inflammatory [8]. A

previous study by Pegoraro et al. (2021) found that tea from *B. pilosa* L. leaves had a nephroprotective effect in protecting rat kidneys from CCl4-induced damage [9]. The present study aimed to determine the nephroprotective effect of *B. pilosa* L. leaf extract on the histological appearance of the kidneys of Wistar rats induced with formalin.

Materials and Methods

Preparation of *B. pilosa* L. Leaf Extract

Plant material obtained from Ngoresan, Jebres, Surakarta, then identified and authenticated at the Department of Biology, Sebelas Maret University, Indonesia (No. 159/UN27.9.6.4/Lab/2024). *B. pilosa* L. leaf powder macerated with 70% ethanol (1:5, w/v) for 3×24 h. The macerate was filtered and concentrated with a rotary evaporator at 55°C [10].

Treatment of Animal Tests

All animal treatments approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Muhammadiyah Surakarta, Indonesia (4809/A.1/KEPK-FKUMS/VII/2023). A total of 25 male Wistar rats (2-3 months old, weighing 200 g) were divided into five treatment groups: control group (Group P0), negative control (Group P1), *B. pilosa* L leaf extract treatment orally with three dose variations of 25 mg/kg BW (Group P21), 50 mg/kg BW (Group P22), and 100 mg/kg BW (Group P23). All animals, except for those in Group P0, received per oral formalin at a dosage of 0.2 ml/kg BW/day for 7 days. Subsequently, the *B. pilosa* L leaf extract was given orally at 25 mg/kg BW (Group P21), 50 mg/kg BW (Group P22), and 100 mg/kg BW (Group P23) for 7 days. The termination of the rats on day 15 was conducted for renal collection. The kidneys were weighed, and histological slides were prepared for examination.

Histology Analysis

Kidney histology was performed using the paraffin technique with Haematoxcillin-Eosin (HE) staining. The slides were observed at 100x and 400x magnification in five randomized fields of view. The abnormal alterations/injuries counted included cell atrophy/dilation, cell degeneration, cell inflammation/fibrosis, and cell necrosis. The scoring system used was according to Table 1 [11].

Table 1. Kidney Profile Score

Score	Kidney Profile Score
1	Abnormal cells <25% of the total visual field
2	Abnormal cells 25≤50% of the total visual field
3	Abnormal cells 50≤75% of the total visual field
4	Abnormal cells >75% of the total visual field

Molecular Docking

Molecular docking was performed on eight active compounds that have been detected in *B. Pilosa* L. [8]. The compounds were retrieved from 3D SDF format through PubChem. The target proteins employed were KEAP1 with PDB ID 7Q96 and TNF-α with PDB ID 2AZ5 to predict antioxidant and anti-inflammatory activities. The interactions were performed using PyRx 0.8, along with visualization using Discovery Studio v16.

Data Analysis

Quantitative data were analyzed using the SPSS (version 25) software with a one-way ANOVA test. If there was a significant difference between treatments, it was followed by Least Significance Different (LSD) with a significance level of 5% (P=0.05).

Results

Effect of *B. pilosa* L. Leaf Extract on Kidney Weight of Rats Due to Formalin Exposure

The results related to kidney weight indicated that the kidney weight was not significantly different between each treatment (Figure 1). This study revealed that administration of formalin 0.2 ml/kg BW/day for 7 days and *B. pilosa* L. leaf extract did not significantly affect the kidney weight in rats (P-value>0.05 in both kidneys).

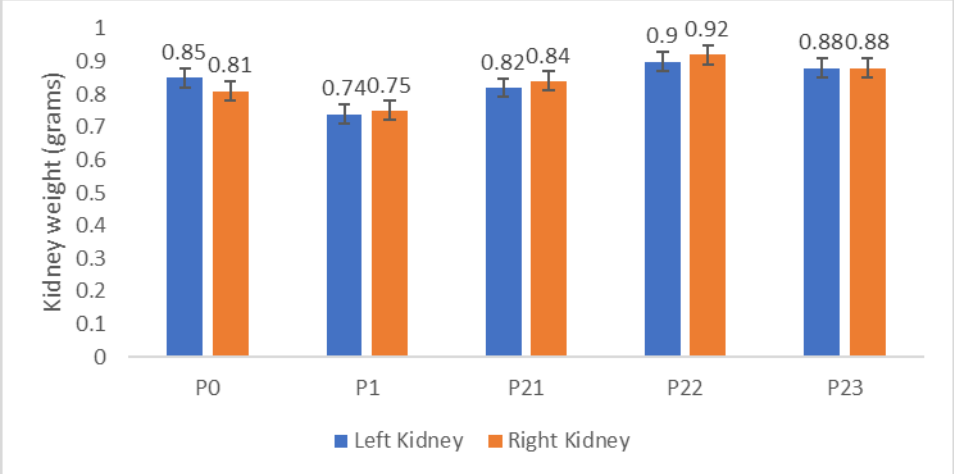

Figure 1. Comparison of the average weight of rat kidneys after *B. pilosa* L. leaf extract treatment. Blue represents the left kidney, while orange represents the right kidney

Effect of *B. pilosa* L. Leaf Extract on the Histological Structure of Rat Kidney Due to Formalin Exposure

Histological observations demonstrated cell degeneration and necrosis in the kidney cells that received formalin. Parenchymatous degeneration cells, hydropic degeneration cells, dilated tubules, and necrosis cells were found in the formalin treatment (Figure 2). The scoring results of microscopic observation showed significant differences in the treatments (Table 2). Formalin treatment in Group P1 increased the number of abnormal cells, as indicated by the high average score of 2.8. Significantly decreased average scoring occurred in Group P23 or the formalin group with *B. pilosa* L. extract with a score of 1.2. In addition, the recovery effect of *B. pilosa* L. extract induction is indicated in Figure 3 by decreasing the amount of degenerated and necrotized cells.

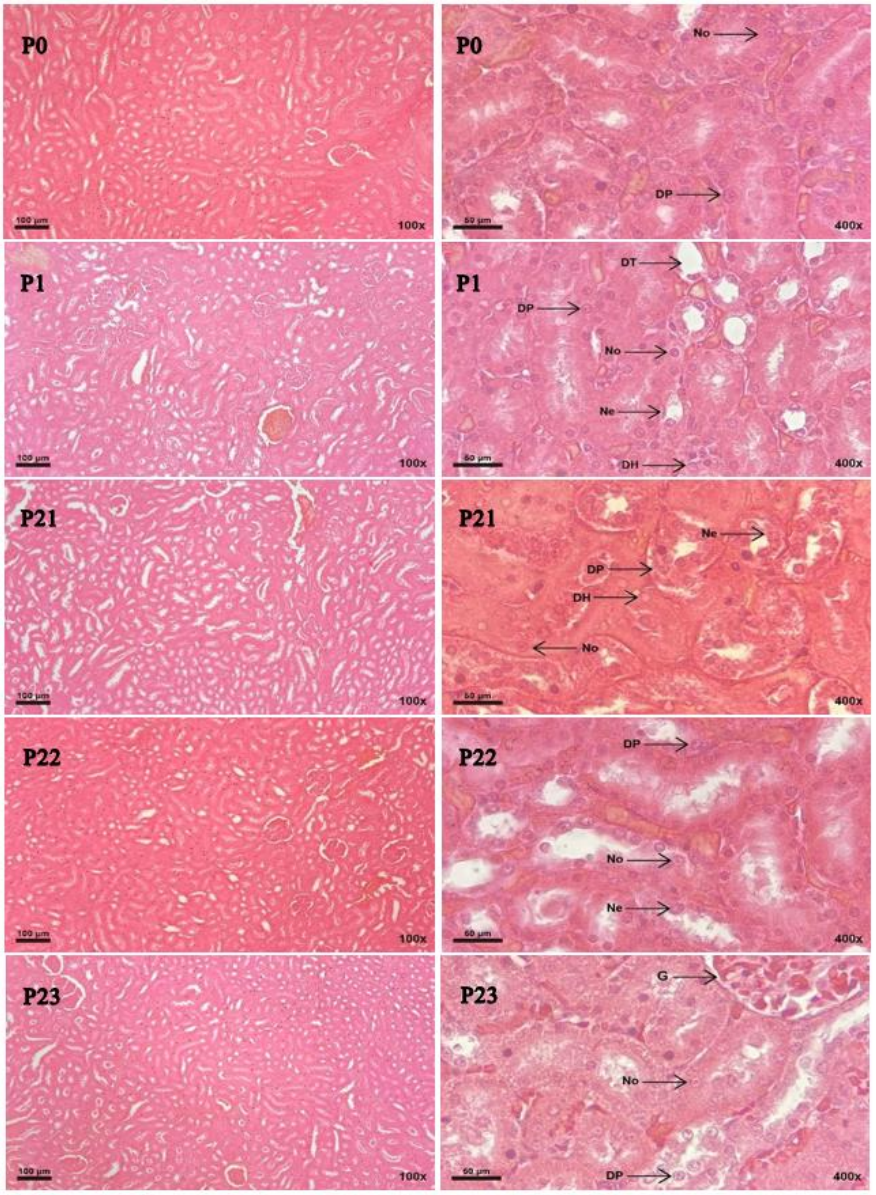

Figure 2. Histology of white rat kidney after treatment with HE stains at 100x and 400x magnification. G: Glomerulus, No: normal kidney cells, DP: parenchymatous degeneration, DH: hydropic degeneration, DT: tubule dilatation, and Ne: necrosis cells

Table 2. Scoring results of kidney cell damage in each treatment.

Group	Mean Percentage of Abnormal Cells (%)	Mean Score
P0	2.1±0.01	1±0.00^{a}
P1	56.5±0.16	2.8±0.45^{b}
P21	54±0.26	2.6±0.89^{bc}
P22	38±0.29	2.2±1.00^{c}
P23	20.5±0.26	1.2±0.89^{a}

Note: a, b, and bc superscripts of the same letter indicate no significant difference from the LSD test results with a significance of P<0.05.

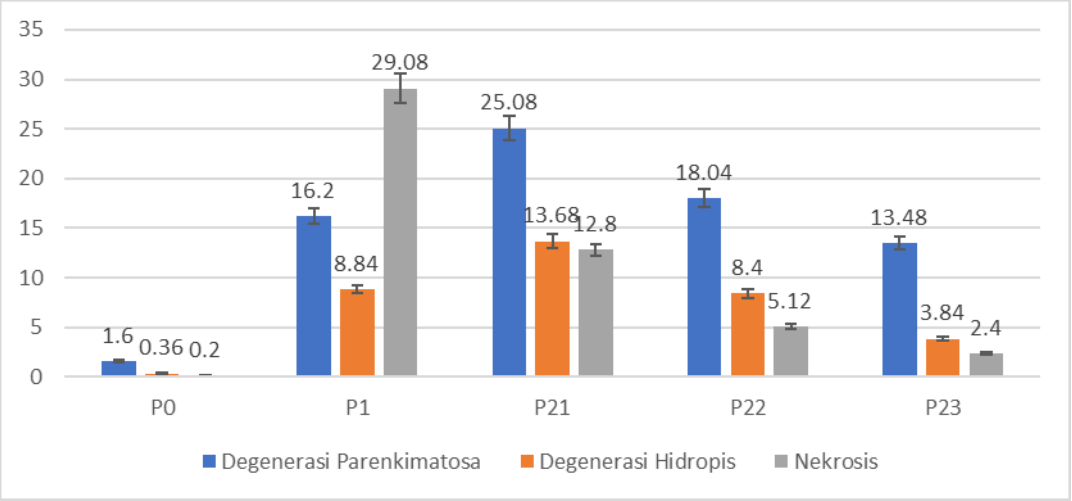

Figure 3. Comparison of the average number of abnormal cells in formalin-induced rat kidneys after treatment with *B. pilosa* L. leaf extract

Molecular Docking of *B. pilosa* L. Secondary Metabolite Compounds

The results of molecular docking demonstrated the interaction between compounds contained in *B. pilosa* L. with Kelch-like ECH-associated protein (KEAP1) so that these compounds had antioxidant activity (Table 3). The compound with the strongest interaction with KEAP1 protein was isochlorogenic acid, followed by luteolin. Conventional hydrogen bonds involved in the interaction are found at Ser 555, Ser 363, Asn 414, Ile 416, and Leu 365 (Figure 4A).

Compounds in *B. pilosa* L. also had the potential for anti-inflammatory response. It was observed that these compounds have the ability to interact with TNF-α, thereby inhibiting the activity of this protein. The isochlorogenic acid compound had the strongest bond with a binding affinity value of -8.9 kcal/mol (Table 3), involving hydrogen bonds with amino acid residues Leu157, Ala 156, and Tyr 151 in chain A, Leu 120 and Tyr 151 in chain B, as well as Tyr 199 in chain C (Figure 4B).

Table 3. Binding affinity values of compounds with target proteins

Compound	Binding Affinity (Kcal/mol)	
	KEAP1	TNF-α
Native ligan	-11.9	-12.1
Isoclorogenic acid	-9.9	-8.9
Luteolin	-8.9	-8
Chlorogenic acid	-8.7	-8.1
Quercetin	-8.3	-7.7
Caffeic acid	-6.4	-7
Ferulic acid	-6.1	-7.3
P-coumaric acid	-5.9	-7.3
Gallic acid	-6.1	-6.2

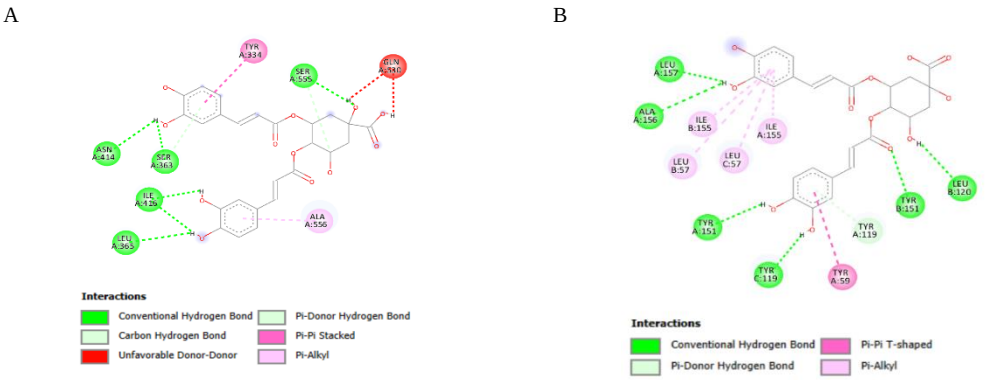

Figure 4. Interaction of isochlorogenic acid compounds on (A) KEAP1 and (B) TNF-α.

Discussion

Formaldehyde is a toxicant to the urinary system, including the kidneys [12]. Intraperitoneal exposure to formalin 10 mg/kg BW for 14 days indicated a significant decrease in kidney weight [13]. However, the 7-day exposure to formalin at a dose of 0.2 ml/kg BW observed in this study revealed no significant change in kidney weight. This lack of change is likely attributed

to the relatively short exposure duration of only seven days and the low dosage, which is below 10 mg/kg BW. Therefore, the exposure was shorter than 14 days, and the dosage was lower than what might typically induce significant effects. Additionally, intraperitoneal exposure is more damaging than oral exposure [14]. Nevertheless, formalin exposure could cause histological damage to the kidneys.

Histological kidney damage showed that the formalin-induced group had a higher damage score than the other groups. The damage included parenchymatous degeneration cells, hydropic degeneration, tubular dilatation, and necrosis. This result is consistent with the research conducted by George et al. (2017), who found tubular dilatation and hydropic degeneration of renal epithelial tubular cells exposed to formaldehyde [15]. More severe damage from formaldehyde exposure for 14 days showed glomerular enlargement, interstitial cell infiltration, and epithelial desquamation to congestion [6]

Kidney histology damage that occurs in formaldehyde-induced groups is a form of cell defense response to formaldehyde exposure and metabolism. Mechanisms exist in healthy cells to rigorously maintain formaldehyde homeostasis through S-adenosylmethionine biosynthesis and one-carbon metabolism [16]. One pathway of the formaldehyde detoxification mechanism is the oxidation reaction by ADH5 on formaldehyde that has reacted with glutathione to form S-formylglutathione, which is metabolized by S-formylglutathione hydrolase into formic acid and water [17]. The metabolism of formaldehyde results in an oxidative stress response due to formaldehyde reacting with glutathione to change the GSH: GSSH ratio [18]. Formaldehyde degradation can occur via glutathione-independent aldehyde dehydrogenase 2, resulting in the final product of formic acid. Additionally, the enzyme catalase, in conjunction with glyoxalase II, also contributes to the degradation process, leading to the final products of water and carbon dioxide [19]. The carbon dioxide produced will be excreted through the respiratory system, while formic acid will be excreted through the urine [3].

Formic acid has inhibited mitochondrial cytochrome c oxidase, thereby reducing ATP synthesis. Acidosis due to formic acid can increase the formation of superoxide anions and hydroxyl radicals, which results in membrane damage, lipid peroxidation, and mitochondrial damage. The decrease in pH also allows calcium to be influx into the mitochondria, which causes mitochondrial dysfunction and cell death [20,21]. Adenosine triphosphate (ATP) deficiency results in hypoxia and loss of control over sodium-potassium ion pumps, causing degenerative changes with cell swelling [22]. Hydropic degeneration is characterized by an increase in the volume of water in the cytosol, while parenchymatous degeneration or cloudy swelling is characterized by granules in the cytoplasm so that it looks cloudy accompanied by cell swelling [23]. Continued cell degeneration causes plasma membrane rupture or necrosis, which promotes cell inflammation [22]. The formalin-induced group was dominated by necrosis cell damage, while all *B. pilosa* L. extract treatment groups were dominated by degeneration cell damage compared to necrosis cells, which indicates that the extract given plays a role in preventing irreversible damage and is able to maintain cell hydrogenation conditions.

The components of *B. pilosa* L. that are extracted with 70% ethanol maceration include flavonoids (e.g., luteolin and quercetin), aromatics (e.g., gallic acid), and phenylpropanoids (e.g., p-coumaric acid, ferulic acid, caffeic acid, chlorogenic acid, and isochlorogenic acid) [8,24]. According to the results of *in silico* tests, these compounds have antioxidant and anti-inflammatory activities. Isochlorogenic acid demonstrated the strongest activity compared to other compounds. These compounds can bind to KEAP1, thereby inhibiting its interaction with NRF2. This inhibition enables the activation of NRF2, which plays a crucial role in regulating the antioxidant response [25]. The anti-inflammatory response is also obtained from the inhibition of TNF-α protein. Inhibition of TNF-α binding to its receptor inhibits NF-κB signaling that regulates pro-inflammatory expression [26]. Flavonoids are also known to act as antioxidants in the kidney by reducing the expression of nitric oxide synthase (iNOS) in the kidney and reducing myeloperoxidase (MPO) activity. The iNOS synthesizes nitric oxide, which is a free radical and can react with superoxide that can damage cell DNA. Then, flavonoids activate the nuclear factor erythroid 2 (Nrf2) defense pathway, which is a transcription factor in encoding the expression of antioxidant enzymes and cytoprotective proteins. This pathway produces proteins and enzymes important in detoxifying and fighting ROS. In addition, flavonoids have anti-apoptotic properties mediated by increased levels of Bcl2, which decreases the Bax/Bcl2 ratio, thereby preventing the induction of apoptosis [27,28].

Conclusions

In conclusion, oral administration of *B. pilosa* L. leaf extract, at the most optimal dose of 100 mg/kg BW, reduced the damage of rat kidney cells caused by formalin exposure. Isochlorogenic acid and luteolin are two secondary metabolite compounds from *B. pilosa* L. that potentially could turn on NRF2 signaling for antioxidant expression and stop inflammation by blocking NF-κB signaling.

Conflict of Interests

The authors declare no conflicts of interest.

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Compliance with Ethical Guidelines

Compliance with ethical guidelines: All animal treatments approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Muhammadiyah Surakarta (4809/A.1/KEPK-FKUMS/VII/2023).

Authors' Contributions

MFA methodology, data analysis and interpretation of results. OPA and WMR article structuring and writing. OPA revision and supervision. MFA and SL analysis and scoring of the kidney damages. All authors have read and approved the manuscript prior to submission for publication.

References

1. Pandey CK, Agarwal A, Baronia A, Singh N. Toxicity of ingested formalin and its management. Hum Exp Toxicol. 2000;**19**(6):360-366. [**doi**:10.1191/096032700678815954] [**pmid**: 10962510]
2. Rahman MB, Hussain M, Kabiraz MP, Nordin N, Siddiqui SA, Bhowmik S, et al. An update on formaldehyde adulteration in food: sources, detection, mechanisms, and risk assessment. Food Chem. 2023; **427**:136761. [**doi**: 10.1016/j.foodchem.2023.136761] [**pmid**: 37406446]
3. Ungureanu LB, Ghiciuc CM, Amalinei C, Ungureanu C, Petrovici CG, Stănescu RȘ. Antioxidants as protection against reactive oxygen stress induced by formaldehyde (FA) exposure: A systematic review. Biomedicines. 2024;**12**(8):1820. [**doi**:10.3390/biomedicines12081820] [**pmid**: 39200284]
4. Reingruber H, Pontel LB. Formaldehyde metabolism and its impact on human health. Curr Opin Toxicol. 2018; **9**:28-34. [**doi**:10.1016/j.cotox.2018.07.001]
5. Dorokhov YL, Shindyapina AV, Sheshukova EV, Komarova TV. Metabolic methanol: molecular pathways and physiological roles. Physiol Rev. 2015;**95**(2):603-644. [**doi**: 10.1152/physrev.00034.2014] [**pmid**: 25834233]
6. Aydemir S, Akgun SG, Beceren A, et al. Melatonin ameliorates oxidative DNA damage and protects against formaldehyde-induced oxidative stress in rats. Int J Clin Exp Med. 2017;**10**(4):6250-6261. [Link]
7. Xuan TD, Khanh TD. Chemistry and pharmacology of Bidens pilosa: an overview. J Pharm Investig. 2016;**46**(2):91-132. [**doi**: 10.1007/s40005-016-0231-6] [**pmid**: 32226639]
8. Bartolome AP, Villaseñor IM, Yang WC. Bidens pilosa L. (Asteraceae): Botanical properties, traditional uses, phytochemistry, and pharmacology. Evid Based Complement Alternat Med. 2013; **2013**:340215. [**doi**: 10.1155/2013/340215] [**pmid**: 23935661]
9. Pegoraro CMR, Nai GA, Garcia LA, Serra FM, Alves JA, Chagas PHN, et al. Protective effects of *Bidens pilosa* on hepatoxicity and nephrotoxicity induced by carbon tetrachloride in rats. Drug Chem Toxicol. 2021;**44**(1):64-74. [**doi**: 10.1080/01480545.2018.1526182] [**pmid**: 30394117]
10. Falowo AB, Muchenje V, Hugo CJ, Charimba G. In vitro antimicrobial activities of Bidens pilosa and Moringa oleifera leaf extracts and their effects on ground beef quality during cold storage. *CyTA - J Food*. 2016;**14**(4):541-546. [**doi**:10.1080/19476337.2016.1162847]
11. Hao X, Luan J, Jiao C, Ma C, Feng Z, Zhu L, et al. LNA-anti-miR-150 alleviates renal interstitial fibrosis by reducing pro-inflammatory M1/M2 macrophage polarization. Front Immunol. 2022; **13:913007**. [**doi**:10.3389/fimmu.2022.913007] [**pmid**: 35990680]
12. Inci M, Zararsız I, Davarci M, Gorur S. Toxic effects of formaldehyde on the urinary system. Türk J Urol. 2013;39(1):48-52. [**doi**: 10.5152/tud.2013.010] [**pmid**: 26328078]
13. Abdel Hamid W, ElMoslemany A, Zeima N. The potential protective effects of aqueous extracts of some herbs on renal toxicity induced by formaldehyde in experimental rats. Bulletin of the National Nutrition Institute of the Arab Republic of Egypt. 2022;**60**(2):1-31. [**doi**:10.21608/bnni.2022.255461]
14. Shoyaib AA, Archie SR, Karamyan VT. Intraperitoneal route of drug administration: should it be used in experimental animal studies? Pharm Res. 2020;**37**(1):12. [**doi**:10.1007/s11095-019-2745-x] [**pmid**: 31873819]
15. George S, Yassa H, Hussein H, El Refaiy A. Protective effect of L- carnitine against formaldehyde-induced kidney, liver and testicular damage in rabbits, a histopathological study. Mansoura J Forensic Med Clin Toxicol. 2017;**25**(2):13-24. [**doi**:10.21608/mjfmct.2018.47239]
16. Pham VN, Bruemmer KJ, Toh JDW, Ge EJ, Tenney L, Ward CC, et al. Formaldehyde regulates *S* -adenosylmethionine biosynthesis and one-carbon metabolism. Science. 2023;**382**(6670): eabp9201. [**doi**: 10.1126/science.abp9201] [**pmid**: 37917677]
17. Nakamura J, Holley DW, Kawamoto T, Bultman SJ. The failure of two major formaldehyde catabolism enzymes (ADH5 and ALDH2) leads to partial synthetic lethality in C57BL/6 mice. Genes Environ. 2020;**42**(1):21. [**doi**: 10.1186/s41021-020-00160-4] [**pmid**: 32514323]
18. Umansky C, Morellato AE, Rieckher M, Scheidegger MA, Martinefski MR, Fernández GA, et al. Endogenous formaldehyde scavenges cellular glutathione resulting in redox disruption and cytotoxicity. Nat Commun. 2022;**13**(1):745. [**doi**:10.1038/s41467-022-28242-7] [**pmid**: 35136057]
19. Kou Y, Zhao H, Cui D, Han H, Tong Z. Formaldehyde toxicity in age-related neurological dementia. Ageing Res Rev. 2022; **73**:101512. [**doi**: 10.1016/j.arr.2021.101512] [**pmid**: 34798299]
20. Liesivuori J, Savolainen H. Methanol and formic acid toxicity: biochemical mechanisms. Pharmacol Toxicol. 1991;**69**(3):157-163. [**doi**: 10.1111/j.1600-0773.1991.tb01290.x] [**pmid**: 1665561]
21. Pietzke M, Meiser J, Vazquez A. Formate metabolism in health and disease. Mol Metab. 2020; **33:23**-37. [**doi**: 10.1016/j.molmet.2019.05.012] [**pmid**: 31402327]
22. Miller MA, Zachary JF. Mechanisms and morphology of cellular injury, adaptation, and death. Pathologic Basis of Veterinary Disease. Elsevier; 2017: 2-43.e19. [**doi**: 10.1016/B978-0-323-35775-3.00001-1]
23. Ilyas S, Hutahaean S, Elimasni, Ar-roisyi DK. Effect of turmeric rhizome extract (*Curcuma longa* L.) on liver histology of preeclampsia rat (*Rattus norvegicus* L.). IOP Conf Ser Earth Environ Sci. 2019;**305**(1):012077. [**doi**:10.1088/1755-1315/305/1/012077]
24. Silvia R, Wahyuni WT, Rohaeti E, Aisyah S, Anggraini Septaningsih D, Hudatul Karomah A, et al. LC-HRMS-based metabolomics approach reveals antioxidant compounds from Centella asiatica leaves extracts. Indonesian Jo Chem. 2024;**24**(6):1861. [**doi**:10.22146/ijc.90782]
25. Crisman E, Duarte P, Dauden E, Cuadrado A, Rodríguez-Franco MI, López MG, et al. KEAP1-NRF2 protein–protein interaction inhibitors: Design, pharmacological properties and therapeutic potential. Med Res Rev. 2023;**43**(1):237-287. [**doi**:10.1002/med.21925] [**pmid**: 36086898]
26. Rasmi RR, Sakthivel KM, Guruvayoorappan C. NF-κB inhibitors in treatment and prevention of lung cancer. Biomed Pharmacother. 2020; **130**:110569. [**doi**: 10.1016/j.biopha.2020.110569] [**pmid**:32750649]
27. Alsawaf S, Alnuaimi F, Afzal S, Thomas RM, Chelakkot AL, Ramadan WS, et al. Plant flavonoids on oxidative stress-mediated kidney inflammation. Biology (Basel). 2022;**11**(12):1717. [**doi**:10.3390/biology11121717] [**pmid**: 36552226]
28. Papi S, Ahmadizar F, Hasanvand A. The role of nitric oxide in inflammation and oxidative stress. Immunopathologia Persa. 2019;**5**(1): e08. [doi:10.15171/ipp.2019.08]