Indonesian Journal of Pharmacy

VOL 32 (2) 2021: 150–157 | RESEARCH ARTICLE

# Acute and Sub-Chronic Toxicity Study of 1-(2, 5-Dihidroxyphenil)-3-Pyridine-2-Il-Propenone In Adult Female Mice

**Ika Puspitasari[1], Ratna Asmah Susidarti[2], Arief Nurrochmad[1], Pridiyanto[3], Devie Amalia Utami[3], Pipit Febrian[3], Thoriq Ziyad[3]**

1. Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy Universitas Gadjah Mada, Skip Utara 55281 Yogyakarta Indonesia
2. Department of Pharmaceutical Chemistry, Faculty of Pharmacy Universitas Gadjah Mada, Skip Utara 55281 Yogyakarta Indonesia
3. Undergraduate student, Faculty of Pharmacy Universitas Gadjah Mada, Skip Utara 55281 Yogyakarta Indonesia

**Info Article**

**Submitted:** 04-11-2020
**Revised:** 09-02-2021
**Accepted:** 05-02-2021

*Corresponding author

Email:
ika.puspitasari@gmail.com

**ABSTRACT**

This research aimed to evaluate the toxicity of 1-(2, 5-dihidroxyphenil)-3-pyridine-2-il-propenone (DPP) after 24h and 90-day administration in female mice. Acute toxicity test was performed using the OECD 423 method, and DPP was administered once a day at doses of 300, 2000, and 5000 mg/kg body weight (BW). Toxic symptoms were observed after 24h of administration and continued until the 14th day. The experimental animals were dissected and examined for histological organs on the 15th day. The sub-chronic toxicity test was performed using the OECD 408 method, and DPP at 14, 28, and 56 mg/kg/day was administered for 90 days. Toxic symptoms were observed every day, and the amount of food and water intakes were also measured. Furthermore, statistical analysis was performed, and changes in body weight, as well as routine blood checks and biochemistry, were observed. At the end of the study, experimental animals were sacrificed, and the vital organs' weights were examined before the histological analysis. The results showed that DPP at 300-5000 mg/kg/day and 14-56 mg/kg/day for 90 days did not show any toxic symptoms respectively. In the sub-chronic toxicity test, no change was observed in blood and urine biochemical parameters ($p \geq 0.05$). However, lymphocytic infiltration in the liver and congested vessel in the kidney occurred after administration at 56 mg/kg/day. The results showed that the acute toxicity of DPP is at category 5 according to Globally Harmonized Classification System and sub-chronic toxicity is at a dose below 56 mg/kg/day.

**Keywords:** acute toxicity, chalcone analog, sub-chronic toxicity

## INTRODUCTION

Chalcones, or 1, 3-diaryl-2-propen-1-ones are flavonoid compounds that are naturally formed from the amino acid phenylalanine. 6-hydroxychalcones are substrates for the biosynthesis of flavonoids such as flavanones, flavonols, flavones, and anthocyanins. Meanwhile, as a 5-deoxyflavonoids precursor, 6-hydroxychalcone is only obtained from Leguminosae plants. Chalcones have antibacterial, antifungal, antiviral, antiprotozoal, anti-cancer, antidiabetic, cardioprotective, neuroprotective, antioxidant, and anti-inflammatory activities (Rozmer and Perjési, 2016).

Chalcone compounds inhibit arachidonic acid (Lin *et al*., 1997), COX-2, NO, and TNF-α (Suzuki *et al*., 2005), and in *in-vitro* studies, the compound 2', 5'-dihydroxychalcone has a strong activity to inhibit cyclooxygenase enzyme ($IC_{50}$ = 37.5μM) compared with other dihydroxichalcones (Lin *et al*., 1997). Some chalcone-derived compounds with anti-inflammatory activity are 2´, 4´-dihydroxychalcone, 4´-hydroxychalcone, and 2´, 5´, - hydroxychalcone (Lin *et al*., 1997; Zhang *et al*., 2010). The 2', 4'-dihydroxychalcone compounds have high anti-inflammatory activity compared to other 12 chalcones derivatives, and at a dose of 200mg/kg, it inhibited swelling in the Kunming

mice ears, which was induced by 62-68% and 53% of xylene and ibuprofen, respectively (Zhang *et al*., 2010). Furthermore, chalcone inhibited prostaglandin E2 (PGE2) (Nowakowska, 2007).

In addition, its anti-inflammatory mechanism and derivatives are through various pathways, such as cyclooxygenase, inhibition of nitric oxide production, and TNF-α, lipoxygenase (*LOX*), interleukin (*IL*), prostaglandin (*PGs*), leukotriene D4 (*LTD4*), nuclear factor-κB (*NF-κB*), intracellular (*ICAM*-1) and vascular cell adhesion molecule-1 (*VCAM*-1), monocyte chemo-attractant protein-1 (*MCP*-1), and TLR4 / MD-2 (Mahapatra *et al*., 2017). This shows the potential for acute and chronic inflammatory therapy. Because compounds which anti-inflammatory activity such as non-steroid anti-inflammatory drugs (NSAIDs) have shown to cause gastrointestinal disorders, it is necessary to test the chalcone safety when used for a long time. DPP is a hydroxychalcone compound that inhibits the inflammation of carrageen-induced rat paw edema at a dose of 200 mg/kg. It inhibits inflammation by 50% equivalent to ibuprofen (Wibowo, 2013).

## METHODS AND MATERIALS

### Preparation of DPP

The synthesis of the DPP compounds was performed according to (Wibowo, 2013) with pyridine-2-carbaldehyde and 2, 5-dihydroxyacetophenone compounds in the microwave and a $K_2CO_3$ catalyst in the absence of solvent.

### Animals and treatment

All the animals used were handled in accordance with the ethical committee of the Animal Ethics, Integrated Laboratory, Gadjah Mada University (UGM), Yogyakarta, Indonesia, number 281/KEC-LPPT/VI/2015 and 31/04/LPPT/IV/2017.

In this study, 78 adult female mice weighed 20-25 g were used, and they were placed in standard mouse cages, maintained under standard conditions (12h light/dark cycle; 25±3°C temperature; 70-80 relative humidity), as well as provided a standard laboratory feed and water *ad libitum*. The drug was administered through oral intubation.

### Dose and duration of acute toxicity treatment

The experimental animals were divided into 4 groups with 6 mice in each. The daily dose of DPP was freshly dissolved in standard volume 0.1mL of 0.5% of sodium carboxymethyl cellulose (CMC) and was orally administered once a day. Group 1 control mice received 0.1mL of 0.5% sodium CMC; Group 2 was treated with DPP at a dose of 300 mg/kg/day; Group 3 was treated with DPP at a dose of 2000 mg/kg/day; Group 4 was treated with DPP at a dose of 5000 mg/kg/day (Pridiyanto, 2016).

### Acute toxicity evaluation

The initial, 2-day, and final body weights were recorded, and parameters indicating toxicity such as the change in the skin, drowsiness, sedation, diarrhea, eye color, tremor, convulsion, salivation, lethargy, and mortality were evaluated at 0.5, 1, 2, 6 and 24h after treatment (OECD, 2001). Toxic symptoms were observed for up to 14 days. Surgery was immediately performed for histological organ observation once death was recorded (Pridiyanto, 2016).

### Dose and duration of sub-chronic toxicity treatment

The experimental animals were divided into 5 groups with 10 mice in each. The daily dose of DPP was freshly dissolved in 0.1mL of 0.5% sodium CMC and was orally administered to each of them every morning at 8 a.m. for 90 days (OECD, 1998). Group A control mice received 0.1mL of 0.5% sodium CMC; Group B was treated with DPP at a dose of 14 mg/kg/day; Group C was treated with DPP at a dose of 28 mg/kg/day (This is a dose conversion from rat to mouse that showed anti-inflammatory activity in a previous study (Wibowo, 2013); Group D were treated with DPP at a dose of 56 mg/kg/day; Group E was the recovery of 105 days after treatment from Group D (satellite group). The animals' initial, weekly and final body weights, as well as food and drink consumption, were recorded (Utami, 2017; Febrian, 2018; Ziyad, 2018).

### Sub-chronic toxicity evaluation

#### Hematological studies

Analysis of red blood cell count (RBC), white blood cell count (WBC), hemoglobin count (Hb), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin count (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, blood sugar, blood cholesterol, blood urea, blood creatinine, as well as aspartate aminotransferase (AST) and alanine transaminase (ALT) was performed using standard methods (Utami, 2017; Ziyad, 2018).

**Organ weight and histopathological examination**

Organs were autopsied under light anesthesia at day 91 and 106. They were dissected and some organs such as stomach, lung, heart, liver, and kidney were excised, cleared from adhering fat and connective tissue using xylene, and then weighed. The organ samples were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5µm using a microtome, and stained with hematoxylin and eosin according to standard laboratory procedures. Thereafter, the stained sections were examined under the microscope for changes in morphology (Febrian, 2018; Ziyad, 2018).

**Statistical analysis**

The data were reported as a mean ± SEM, and one-way ANOVA as well as Tukey test were used for statistical comparison. All values of $p<0.05$ were considered significantly different.

## RESULTS AND DISCUSSION

**Effect of DPP on general appearance and behavioral observation**

The toxicity study of DPP was determined following the guidelines of OECD 423 and 408 respectively. Meanwhile, all the treated groups orally received DPP at doses of 300, 2000, and 5000 mg/kg once per day for acute toxicity study. Furthermore, a sub-chronic toxicity study was performed for 90 days at doses of 14, 28, and 56 mg/kg daily, and no clinical signs were observed in these studies (DPP-treated group compared with the control).

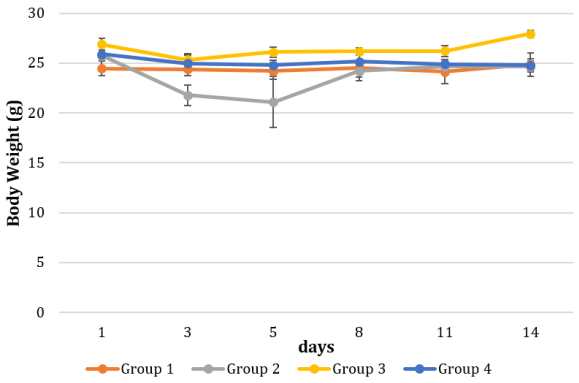


Figure 1. Mean body weight change in mice treated with 300 (group 2), 2000 (group 3), and 5000 mg/kg/d DPP (group 4) as compared with the control group (group 1) during acute toxicity study (P>0.05) (Pridiyanto, 2016).

**Effect of DPP on relative organ body weight**

There was no vital distinction in average organ weight between control and DPP treatment at doses at doses of 300, 2000, and 5000 mg/kg in the acute toxicity (Figure 1). Moreover, in sub-chronic, there was no difference in the average organ weight between the two groups (doses 14-56mg/kg) (Figure 2). Therefore, the results showed that the vital organs such as the stomach, lung, heart, liver, and kidney were not adversely affected throughout the treatment by DPP (P>0.05).

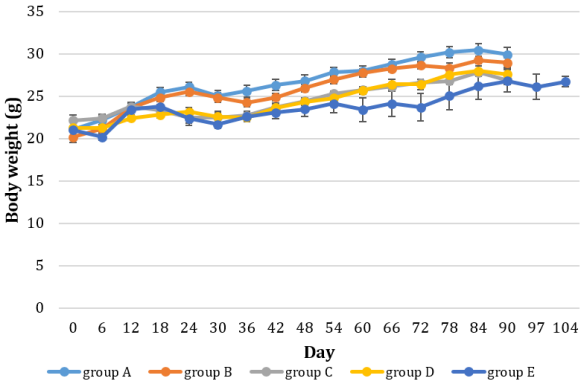


Figure 2. Mean body weight change in mice treated with 14 (group B), 28 (group C), 56 mg/kg/d DPP (group D), and recovery group (group E) as compared to the control group (group A) during sub-chronic toxicity study (P>0.05) (Utami, 2017).

**Effect of DPP on hematological parameters**

All the recorded hematological parameters were within normal values **(Table I).**

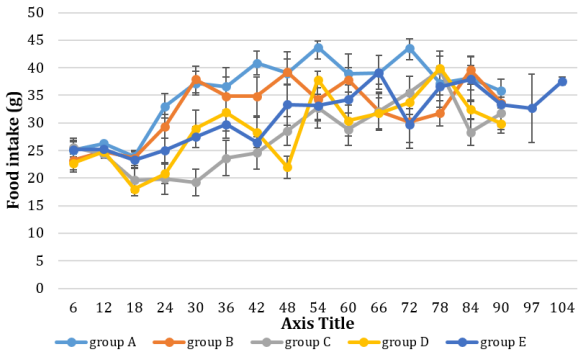


Figure 3. Mean food consumption in mice treated with 14 (group B), 28 (group C), 56 mg/kg/d DPP (group D), and recovery group (group E) as compared to the control group (group A) during sub-chronic toxicity study (P>0.05) (Utami, 2017).

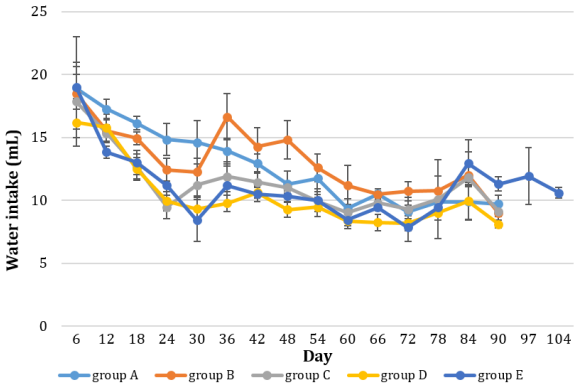


Figure 4. Mean water consumption in mice treated with 14 (group B), 28 (group C), 56 mg/kg/d DPP (group D), and recovery group (group E) as compared to the control group (group A) during sub-chronic toxicity study (P>0.05) (Utami, 2017).

**Effect of DPP on biochemical parameters**

The results of the various biochemical tests (**Table II**), and oral administration of DPP at a dose of 14-56 mg/kg did not cause a significant change in AST, ALT, blood creatinine, blood urea, glucose, and cholesterol levels when compared to the control group (P>0.05). All parameters were in normal range value of biochemical level in mice (Nurrochmad and Airin, 2013).

**Histopathological studies**

There was no abnormality following DPP administration at a dose of 56 mg/kg/d on abdomen, lung, and heart (Figure 5). The histological analysis showed that there was lymphocytic infiltration in the liver and congested vessel in the kidney after 90 days of DPP administration at high dose.

Previously, NSAIDs have been used as anti-inflammatory drugs. However, their side effect includes gastrointestinal toxicity, and in short term, they cause digestion problems such as heartburn, dyspepsia, and nausea or stomach pain. In addition, they have caused gastroduodenal ulcers and gastrointestinal bleeding after prolonged use. Therefore, there is a need to find new anti-inflammatory drugs with no side effects on the digestive tract and other organ disorders. Currently, there are natural and synthesized chalcone compounds that have been studied for their pharmacological activities on various diseases, including anti-inflammatory activities. Furthermore, DPP has been shown to inhibit inflammation, and its anti-inflammatory potential is equivalent to ibuprofen. Thus, developing DPP's acute and sub-chronic toxicity testing is needed, considering its possibility as an anti-inflammatory drug for osteoarthritis treatment used for a long period by patients.

The toxicity test conducted for the initial stage was *in vitro* using *Artemia salina* Leach shrimp larvae or known as the Brine Shrimp Lethality Test (BSLT), and it is mostly performed for initial screening of anticancer compounds. This test is relatively simple, rapid, and does not require special equipment (Meyer *et al.*, 1982). Some chalcone compounds have been tested against BSLT. Perdana *et al.* (2015) synthesized anti-cancer derivatives of methoxychalcones, which were produced from the reaction of 4-methoxy acetophenone and aromatic aldehyde derivatives. Consequently, three para methoxychalcones compounds showed safety, as evidenced by the $LC_{50}$ value <200µg / mL using the BSLT method (Perdana *et al.*, 2015). In this study, acute toxicity of DPP is at category 5 according to the Globally Harmonized Classification System.

In addition, some research use cancer cell lines (Shin *et al.*, 2013) and rat liver epithelial cells to determine the safety of chalcone compounds (Forejnikova *et al.*, 2005). Similarly, human hepatic stellate cells were used to test some chalcone compounds toxicity, and studies have established a link between the different structures of the 19 tested compounds. The Michael-system structure has a significant impact on proliferation, mitochondrial mass, and cytochrome c secretion. Therefore ring A substitution will increase both chalcone activity and toxicity while the presence of OH in the 6' position reduces their toxicity levels (Zenger *et al.*, 2015). The result of this study showed that sub-chronic DPP administration caused liver disorders as characterized by lymphocytic infiltration (Figure 5), however, no significant change in the ALT and AST parameters was observed (Table II). NSAIDs are known to induce liver toxicity, therefore, they have been withdrawn from circulation (O'Connor *et al.*, 2003). NSAIDs that selectively inhibit COX-2 (celecoxib, rofecoxib) also cause side effects such as hepatotoxicity even when they are not usually serious (Bénichou, 1990). There have been reports on the swelling of the mitochondria of liver cells treated with diphenylamine, mefenamic acid, or diclofenac. The accumulation of diclofenac metabolites and reduced MRP2 expression are thought to be the cause of the liver injury. However, MRP2 is a protein for transporting diclofenac acyl glucuronide to biliary canaliculi (Aithal, 2011).

Table I. Hematological parameters of female mice after DPP administration (Ziyad, 2018)

| Treatment | RBC ($x10^6$/µl) | WBC ($X10^3$/µl) | Hb (g/dl) | Hematocrit (%) | MCV (fl) | MCH (pg) | MCHC (g/dl) | Platelet count ($X10^4$/µl) |
|---|---|---|---|---|---|---|---|---|
| **Group A, control (vehicle-treated)** | 9.51± 0.28 | 7.22± 0.96 | 14.75± 0.36 | 48.84± 1.29 | 51.38± 0.46 | 15.53± 0.30 | 30.24± 0.43 | 116.85± 7.81 |
| **Group B, 14 mg/kg/d for 90 days** | 8.85± 0.57 | 6.39± 1.02 | 13.22± 0.73 | 44.26± 2.67 | 50.14± 0.58 | 15.04± 0.25 | 29.96± 0.30 | 107.68± 15.26 |
| **Group C, 28 mg/kg/d for 90 days** | 9.46± 0.12 | 7.15± 0.74 | 13.98± 0.21 | 47.18± 0.70 | 49.85± 0.40 | 14.78± 0.12 | 29.64± 0.17 | 127.12± 9.64 |
| **Group D, 56 mg/kg/d for 90 days** | 9.30± 0.14 | 7.27± 0.74 | 14.08± 0.31 | 46.89± 0.69 | 50.48± 0.42 | 15.16± 0.31 | 30.02± 0.42 | 110.03± 8.03 |
| **Group E, recovery withdrawal period** | 9.17± 0.22 | 6.17± 0.68 | 14.00± 0.23 | 47.95± 0.65 | 52.30± 0.81 | 15.25± 0.30 | 29.20± 0.24 | 147.20± 13.35 |

Values are stated as a mean ± SEM. P>0.05, when compared to the control group (One-way ANOVA followed by Tukey test), RBC (red blood cell count). WBC (white blood cell count), Hb (hemoglobin count), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin count), MCHC (mean corpuscular hemoglobin concentration).

Table II. Biochemistry parameters of female mice after DPP administration (Utami, 2017)

| Treatment | Blood sugar (mg/dl) | AST (U/l) | ALT (U/l) | Blood creatinine (mg/dl) | Blood urea (mg/dl) | Total cholesterol (mg/dl) |
|---|---|---|---|---|---|---|
| **Group A, control (vehicle-treated)** | 123.73± 10.75 | 134.98± 9.25 | 37.21± 4.59 | 0.17± 0.02 | 53.46± 4.45 | 97.58± 7.25 |
| **Group B, 14 mg/kg/d for 90 days** | 137.20± 10.51 | 119.16± 19.88 | 39.25± 5.56 | 0.17± 0.03 | 51.00± 3.98 | 96.68± 13.38 |
| **Group C, 28 mg/kg/d for 90 days** | 118.26± 7.72 | 102.97± 15.46 | 44.86± 6.72 | 0.15± 0.01 | 50.63± 3.28 | 86.12± 4.27 |
| **Group D, 56 mg/kg/d for 90 days** | 128.56± 8.66 | 121.48± 15.46 | 51.66± 4.71 | 0.15± 0.02 | 47.74± 3.71 | 89.56± 5.14 |
| **Group E, recovery withdrawal period** | 120.68± 6.51 | 124.90± 14.67 | 27.78± 3.64 | 0.18± 0.03 | 41.73± 2.67 | 86.93± 13.00 |

Values are stated as a mean ± SEM. *P≤0.05, when compared to the control group (One-way ANOVA followed by Tukey test).

After a prolonged DPP treatment in this study, it is possible that the metabolite caused liver injury (Pandit *et al*., 2012).

Previous studies have shown that chalcones possessing anti-inflammatory activities include indol-based, methoxy, pyrazole, and morpholine-containing chalcones (Tekale *et al*., 2020). However, these compounds have not been tested for acute or sub-chronic toxicity *in vivo*, therefore their effects on the organs are not known.

The six trimethoxychalcones compounds tested did not show any significant toxicity in all organs/glands of experimental animals (Figueiredo *et al*., 2015). In contrast, this study showed toxicity effect of DPP on the liver and kidneys (Figure 5 and 6). NSAIDs affect the digestive tract and are further known as drugs that cause nephrotoxicity since they cause acute kidney injury by decreasing intra-medullary renal and ischemic perfusion. Furthermore, they increase the risk of acute tubular necrosis and trigger interstitial nephritis. The use of phenoprofen, naproxen and ibuprofen causes nephrotic proteinuria (Lucas *et al*., 2019). The ability of DPP to inhibit COX 1 and 2 plays a role in decreasing renal GFR as well as sodium and water retention. Therefore, it may cause congested vessels in the kidney after its administration at a dose of 56 mg/kg/day (Figure 6), while considering that the treatment is performed within 3 months. The NSAIDs administration causes hepato-renal toxicity. Similarly, diclofenac leads to renal degeneration disturbances (Modi *et al*., 2012).

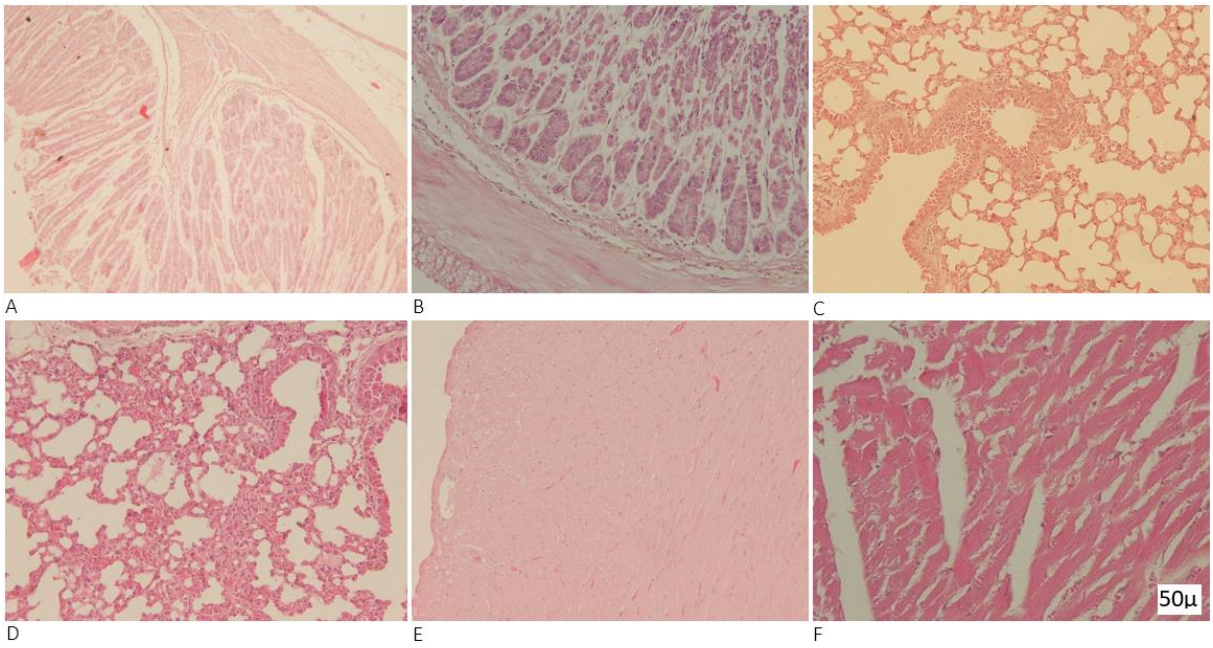


Figure 5. Cross-section of HE stain A. normal B. DPP treatment at a dose of 56 mg/kg/d on mouse stomach; C. normal D. DPP treatment at a dose of 56 mg/kg/d on mouse lung; E. normal F. DPP treatment at a dose of 56 mg/kg/d on the heart (Febrian, 2018)..

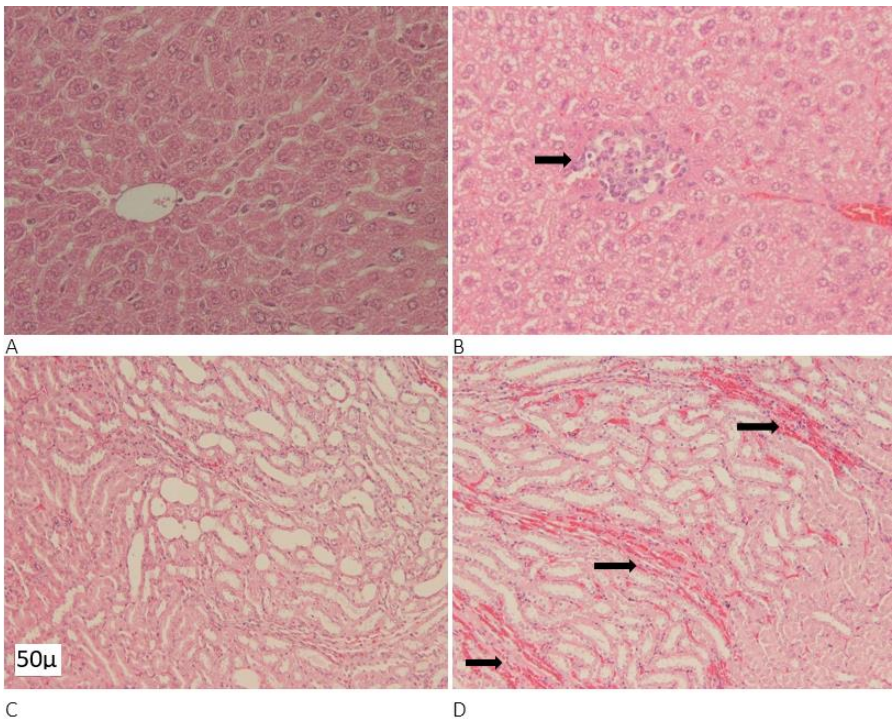


Figure 6. Cross-section of HE stain A. normal B. DPP treatment at a dose of 56 mg/kg/d on mouse liver (arrow=lymphocytic infiltration); C. normal D. DPP treatment at a dose of 56 mg/kg/d on mouse kidney (arrow=congested vessel) (Febrian, 2018; Ziyad, 2018).

Post-marketing studies reported that celecoxib caused acute interstitial nephritis (less than 0.1%), membranous glomerulopathy, as well as papillary necrosis in patients (Mukherjee *et al*., 2001). The results showed that DPP administration at a dose of 56 mg/kg/day for 90 days caused lymphocytic infiltration in the liver and congested vessels in the kidney. Furthermore, its use as an anti-inflammatory drug is still possible at a dose below 56 mg/kg/day following the results of DPP testing at a dose of 28 mg/kg (similar to 200 mg/kg in rat).

## CONCLUSION

Considering the results, it is reasonable to conclude that the acute toxicity of DPP for anti-inflammatory activities in female mice was category 5 according to Globally Harmonized Classification System. DPP administration for 90 days did not show any significant disturbance at a dose below 56 mg/kg/day.

## ACKNOWLEDGEMENTS

The authors are thank to the State Ministry of Research and Technology for financial support under project number 814/UN1-P.III/LT/DIT-LIT/2016. We are also grateful to Andi Eko Wibowo, MSc, for providing DPP and drh. Sitarina Widyarini, MP, Ph.D. for histopathological interpretation.

## CONFLICT OF INTERESTS

All authors declare that there are no conflicts of interest.

## REFERENCES

Aithal, G. P. (2011). Hepatotoxicity related to antirheumatic drugs. *Nature Reviews. Rheumatology*, *7*(3), 139–150. https://doi.org/10.1038/nrrheum.2010.214

Bénichou, C. (1990). Criteria of drug-induced liver disorders. Report of an international consensus meeting. *Journal of Hepatology*, *11*(2), 272–276. https://doi.org/10.1016/0168-8278(90)90124-a

Febrian, P. 2018. Uji toksisitas sub kronis senyawa 1-(2,5- dihidroksifenil)-3-(piridin-2-il) propenon pada mencit betina galur Swiss : studi parameter histopatologi. Skripsi. Fakultas Farmasi, Universitas Gadjah Mada.

Figueiredo, C. P., Ferreira, N. C., Passos, G. F., Costa, R. D., Neves, F. S., Machado, C. S. C., Mascarello, A., Chiaradia-Delatorre, L. D., Neuenfeldt, P. D., Nunes, R. J., and Cordeiro, Y. (2015). Toxicological Evaluation of Anti-Scrapie Trimethoxychalcones and Oxadiazoles. *Anais Da Academia Brasileira de Ciências*, *87*(2), 1421–1434. https://doi.org/10.1590/0001-3765201520140712

Forejnikova, H., Kunerova, K., Kubinova, R., Jankovska, D., Marek, R., R, K., V, S., J, V., and M, M. (2005). Chemoprotective and toxic potentials of synthetic and natural chalcones and dihydrochalcones in vitro. *Toxicology*, *208*(1), 81–93. https://doi.org/10.1016/j.tox.2004.11.011

Lin, C. N., Lee, T. H., Hsu, M. F., Wang, J. P., Ko, F. N., and Teng, C. M. (1997). 2',5'-Dihydroxychalcone as a potent chemical mediator and cyclooxygenase inhibitor. *The Journal of Pharmacy and Pharmacology*, *49*(5), 530–536. https://doi.org/10.1111/j.2042-7158.1997.tb06837.x

Lucas, G. N. C., Leitão, A. C. C., Alencar, R. L., Xavier, R. M. F., Daher, E. D. F., and Silva Junior, G. B. da. (2019). Pathophysiological aspects of nephropathy caused by non-steroidal anti-inflammatory drugs. *Jornal Brasileiro De Nefrologia: 'Orgao Oficial De Sociedades Brasileira E Latino-Americana De Nefrologia*, *41*(1), 124–130. https://doi.org/10.1590/2175-8239-JBN-2018-0107

Mahapatra, D., Bharti, S., and Asati, V. (2017). Chalcone Derivatives: Anti-inflammatory Potential and Molecular Targets Perspectives. *Current Topics in Medicinal Chemistry*, *17*(28), 3146–3169. https://doi.org/10.2174/1568026617666170914160446

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., and McLaughlin, J. L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*, *45*(5), 31–34. https://doi.org/10.1055/s-2007-971236

Mukherjee, D., Nissen, S. E., and Topol, E. J. (2001). Risk of cardiovascular events associated with selective COX-2 inhibitors. *JAMA*, *286*(8), 954–959. https://doi.org/10.1001/jama.286.8.954

Nowakowska, Z. (2007). A review of anti-infective and anti-inflammatory chalcones. *European Journal of Medicinal Chemistry*, *42*(2), 125–137. https://doi.org/10.1016/j.ejmech.2006.09.019

Nurrochmad, A. and Airin, C.M., 2013, Standarisasi Hewan Laboratorium UnitPengembangan Hewan Percobaan dalam Rangka Penguatan RisetLingkungan Universitas Gadjah Mada, Laporan Penelitian, LembagaPenelitian dan Pengabdian Masyarakat Universitas Gadjah Mada,Yogyakarta

O'Connor, N., Dargan, P. I., and Jones, A. L. (2003). Hepatocellular damage from non-steroidal anti-inflammatory drugs. *QJM: Monthly Journal of the Association of Physicians*, *96*(11), 787–791. https://doi.org/10.1093/qjmed/hcg138

OECD. (n.d.-a). *OECD iLibrary | Test No. 423: Acute Oral toxicity—Acute Toxic Class Method*. Retrieved July 21, 2020, from https://www.oecd-ilibrary.org/environment/test-no-423-acute-oral-toxicity-acute-toxic-class-method_9789264071001-en

OECD. (n.d.-b). *Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents* [Text]. Retrieved July 21, 2020, from https://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

Pandit, A., Sacdeva, T., and Bafna, P. (n.d.). *Drug-Induced hepatotoxicity:a review*. https://doi.org/10.7324/JAPS.2012.2723

Perdana, F., Eryanti, Y., and Zamri, A. (2015). Synthesis and Toxicity Assessments Some Para-methoxy Chalcones Derivatives. *Procedia Chemistry*, *16*, 129–133. https://doi.org/10.1016/j.proche.2015.12.040

Pridiyanto. 2016. Uji Toksisitas akut senyawa 1-( 2,5-dihidroksifenil)-3-piridin-

2-il-propenon pada mencit betina galur Balb/c. Skripsi. Fakultas Farmasi, Universitas Gadjah Mada.

Rozmer, Z., and Perjési, P. (2016). Naturally occurring chalcones and their biological activities. *Phytochemistry Reviews*, *15*(1), 87–120. https://doi.org/10.1007/s11101-014-9387-8

Shin, S., Yoon, H., Ahn, S., Kim, D., Kim, K., Koh, D., Lee, Y., and Lim, Y. (2013). Chromenylchalcones showing cytotoxicity on human colon cancer cell lines and in silico docking with aurora kinases. *Bioorganic and Medicinal Chemistry*, *21*(14), 4250–4258. https://doi.org/10.1016/j.bmc.2013.04.086

Suzuki, K., Seung, B. H., Sung, L. S., Sanghyun, L., Hoon, J. S., Sil, L. Y., Hyun, S. K., and Ohuchi, K. (2005). The mechanism of anti-inflammatory activity of 2'-hydroxychalcone derivatives. 炎症・再生, *25*(2), 130–136. https://doi.org/10.2492/jsir.25.130

Tekale, S., Maskele, S., Pooe, O., Kendrekar, P., and Pawar, R. (n.d.). *Biological Role of Chalcones in Medicinal Chemistry | IntechOpen*. Retrieved July 21, 2020, from https://www.intechopen.com/books/vector-borne-diseases-recent-developments-in-epidemiology-and-control/biological-role-of-chalcones-in-medicinal-chemistry

Utami, A.D. 2017. Uji toksisitas subkronis senyawa 1-(2,5-dihidroksifenil)-(3-

piridin-2-il)-propenon pada mencit betina galur Swiss Webster: studi parameter kimia darah. Skripsi. Fakultas Farmasi, Universitas Gadjah Mada.

Wibowo, A.E. 2013. Sintesis dan uji aktifitas antiinflamasi senyawa 1- (2,5-dihidroksifenil)-(3-piridin-2-il)- propenon. Tesis. Program Studi Ilmu Farmasi, Fakultas Farmasi, Universitas Gajah Mada.

Zenger, K., Dutta, S., Wolff, H., Genton, M. G., and Kraus, B. (2015). In vitro structure-toxicity relationship of chalcones in human hepatic stellate cells. *Toxicology*, *336*, 26–33. https://doi.org/10.1016/j.tox.2015.07.012

Zhang, X.-W., Zhao, D.-H., Quan, Y.-C., Sun, L.-P., Yin, X.-M., and Guan, L.-P. (2010). Synthesis and evaluation of antiinflammatory activity of substituted chalcone derivatives. *Medicinal Chemistry Research*. https://agris.fao.org/agris-search/search.do?recordID=US201301825872

Ziyad, T. 2018. Uji toksisitas sub kronis senyawa 1-(2,5- dihidroksifenil)-3-(piridin-2-il) propenon pada mencit betina galur Swiss Webster : kajian parameter hematologi dan fungsi ginjal. Skripsi. Fakultas Farmasi, Universitas Gadjah Mada.

Indonesian Journal of Pharmacy

VOL 32 (2) 2021: 150–157 | RESEARCH ARTICLE